جامعة طنطا – فرع كفر الشيخ.

كلية الطب البيطري.

قسم الطفيليات.

تحت رعاية

السيد **أ.د./ فـؤاد خليـــفة هـراس** - رئيس جامعة طنطا

السيد **أ.د./ حسـن إبـراهيم عبـد** - نائب رئيس جامعة طنطا لشئون فرع كفر الشيخ

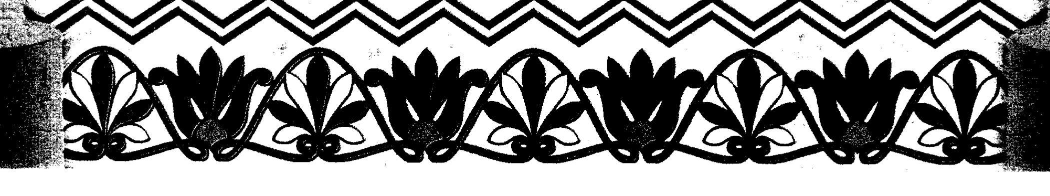
يقيم قسم الطفيليات بكلية الطب البيطرى - بكفر الشيخ

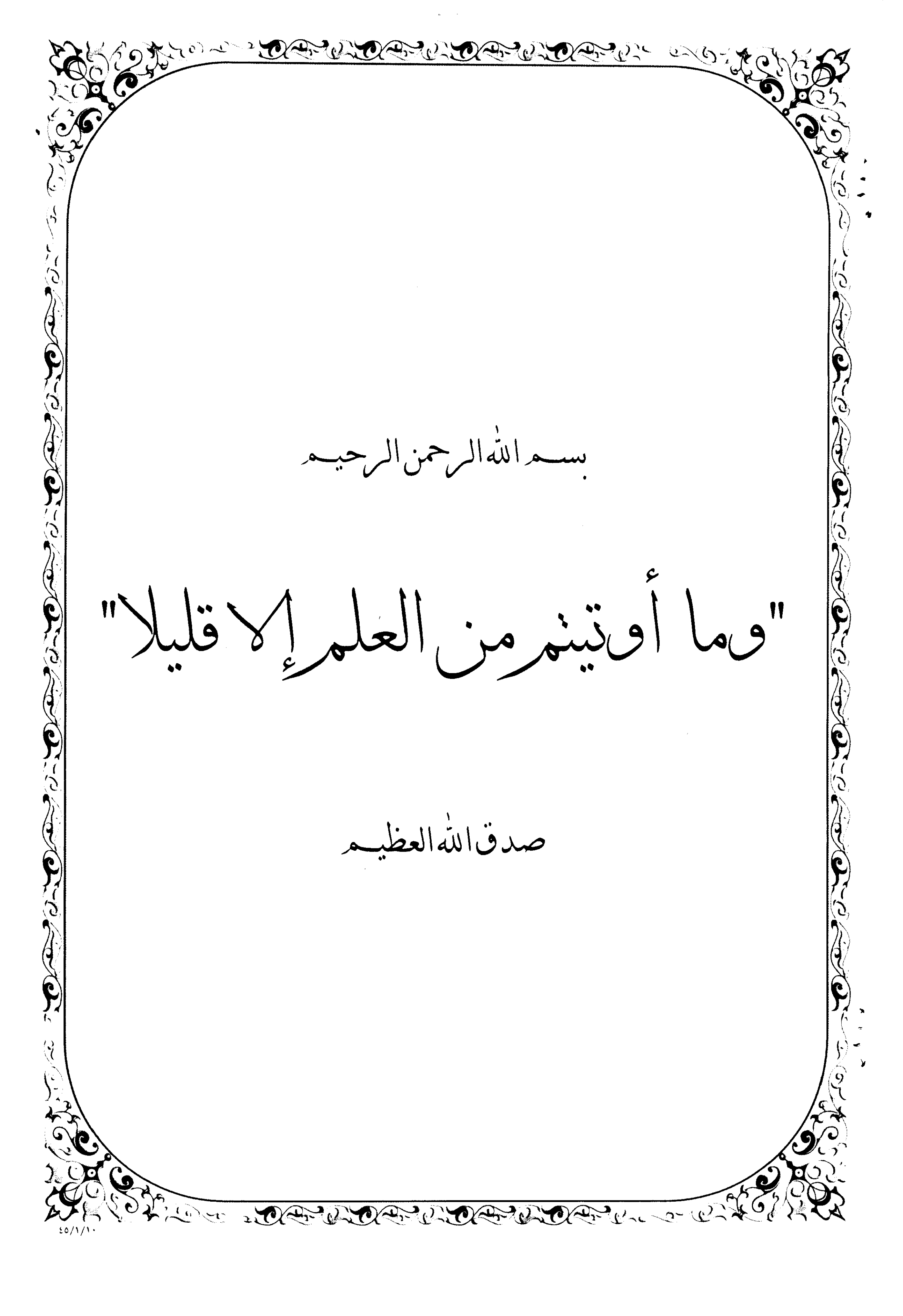
ندوة علمية حول

" طفيليات الدم فى حيوانات المزرعة "

وذلك يوم الأحـــد الموافق ٢٠٠٤/٥/٩ م.

أمين الندوة	مقرر الندوة	رئيس الندوة
د./عبد الرازق يوسف دسوقى	**أ.د/احمد علي السواق**	**أ.د./محمود عبد النبى الصيفى**
أستاذ مساعد الطفيليات -كلية الطب البيطري بكفر الشيخ	وكيل كلية الطب البيطري بكفر الشيخ لشئون خدمة المجتمع و تنمية البيئة	أستاذ و رئيس قسم الطفيليات و عميد كلية الطب البيطري بكفر الشيخ





بسم الله الرحمن الرحيم

"وما أوتيتم من العلم إلا قليلا"

صدق الله العظيم

٤٥/١/١٠

برنامج الندوة

جامعة طنطا – فرع كفر الشيخ

كلية الطب البيطري

قسم الطفيليات

البرنامج	الى	من
وصول السادة الضيوف والتسجيل	١٠ ص	٩:٣٠
الجلسة الافتتاحية	١٠:٣٠ ص	١٠
حفل شاي بقسم الطفيليات	١١:٣٠ ص	١٠:٣٠
المحاضرة الأولى حول "*أضواء بيولوجية على القراد*" **أ.د./ محمود عبد النبى الصيفى** أستاذ و رئيس قسم الطفيليات وعميد كلية الطب البيطرى بكفر الشيخ	١٢:٣٠ ظهراً	١١:٣٠
المحاضرة الثانية حول "*الإصابة بالبابيزيا و الثيليريا فى حيوانات المزرعة*" **د/مجدى حسانين الجعبرى** أستاذ مساعد أمراض الحيوان– قسم طب الحيوان –كلية طب بيطري بكفر الشيخ	١:٣٠	١٢:٣٠
المحاضرة الثالثة حول "*الإصابة بالفيلاريا فى حيوانات المزرعة*" **أ.د./ طاهر محمد عبد الوهاب** رئيس معمل بيطرى –كفر الشيخ	٢:٣٠	١:٣٠
المحاضرة الرابعة حول *الإصابة بالتربانوسوما فى حيوانات المزرعة*" **د/ عبد الرازق يوسف دسوقى** أستاذ مساعد الطفيليات –كلية الطب البيطرى بكفر الشيخ	٣:٣٠	٢:٣٠
المناقشة والتوصيات	٤:٣٠ مساءً	٣:٣٠
غذاء و مغادرة	٤:٣٠	

يوجد أتوبيس لنقل السادة ضيوف الندوة من ميدان روكسي الساعة ٧:٣٠ صباحاً ومن ميدان التحرير بجوار (شركة ساس) الساعة ٨ والعودة إلى القاهرة بعد انتهاء الندوة.

بسم الله الرحمن الرحيم

نبذة عن النشاط العلمى لقسم الطفيليات
كلية الطب البيطرى بكفر الشيخ
جامعة طنطا

أعضاء هيئة التدريس ومعاونيهم بالقسم:

١ – الأستاذ الدكتور / **محمود عبد النبى عمر الصيفى**
أستاذ ورئيس قسم الطفيليات وعميد الكلية

٢ – الدكتور / **عبد الرازق يوسف عبد العزيز دسوقى**
أستاذ مساعد الطفيليات

٣ – ط.ب./ **رضا البسطويسى ابراهيم خلف الله**
مدرس مساعد الطفيليات

٤ – ط.ب./ **شيماء صبحى غريب** معيدة بالقسم

٥ – ط.ب./ **خالد مسعد سلطان** معيد بالقسم

قام القسم بعمل عدد من الندوات العلمية وهى :–

١ – **طفيليات الأسماك** بتاريخ ١٩٩٩/٥/١٧ .
٢ – **الطفيليات المشتركة** بتاريخ ٢٠٠٠/٣/١ .
٣ – **كوكسيديا الطيور** بتاريخ ٢٠٠٠/٧/٢٦ .
٤ – **الديدان الكبدية فى الحيوان والإنسان** بتاريخ ٢٠٠١/١١/١٤ .
٥ – **طفيليات الطيور والأرانب** بتاريخ ٢٠٠٢/٤/٢ .
٦ – **القراد وطفيليات الدم المنقولة لحيوانات المزرعة** بتاريخ ٢٠٠٢/٥/١٥م.
٧ – **طفيليات الدم فى حيوانات المزرعة** بتاريخ ٢٠٠٤/٥/٩م.

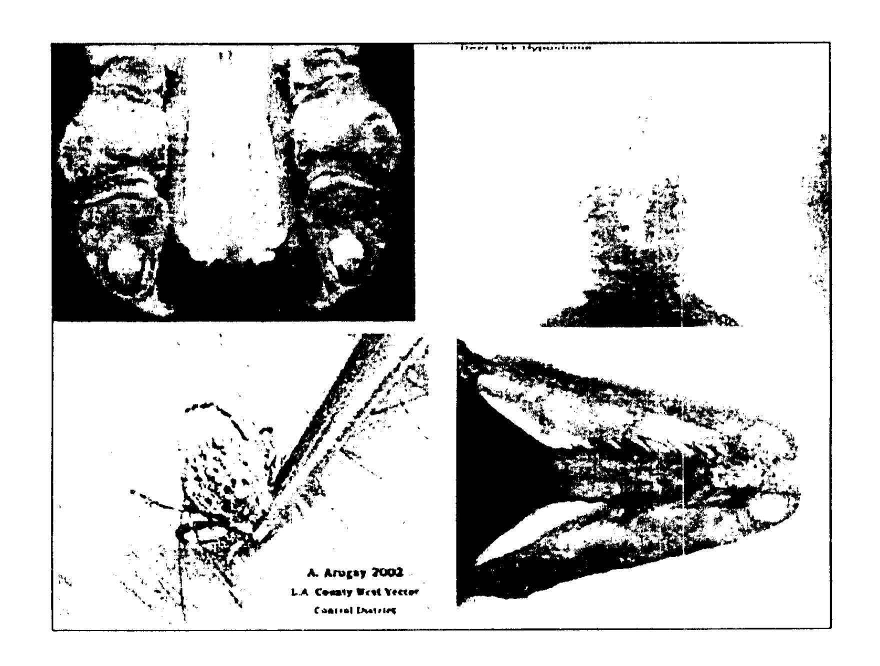
و الله ولى التوفيق

Some Biological Aspects on Hard Ticks

Presented by:

Dr. Mahmoud A. El-seify

Prof. & Chairman of Parasitology Department
Dean of Fac.Vet.Med. Kafr El-Sheikh
Tanta University



Types & Species of Hard Ticks

A- One Host Tick:

e.g. Boophilus

"B.annultus"

" B.microplus"

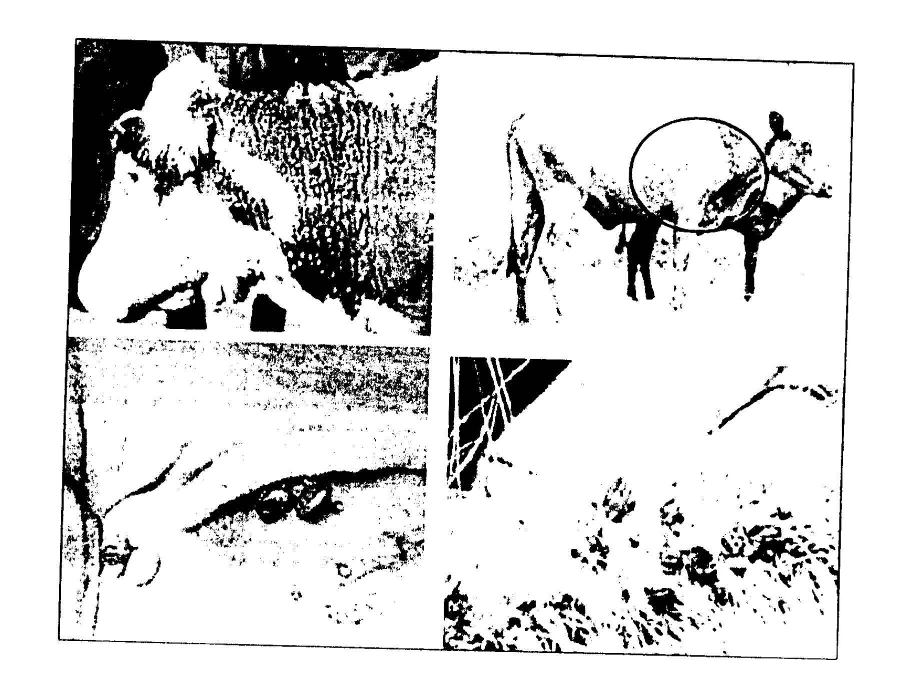


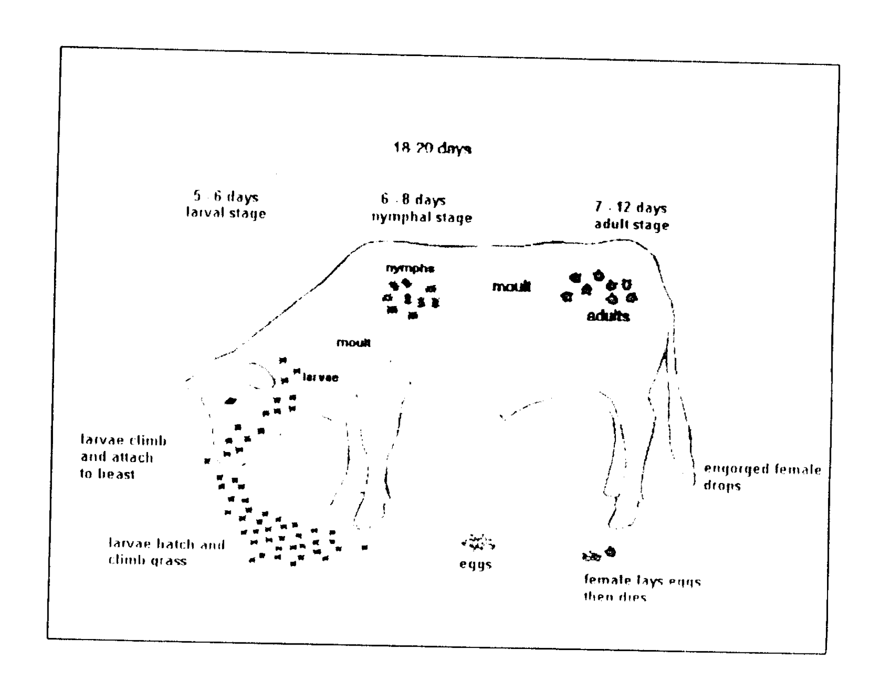
*Infest cattle allover the body (between hairs) during all seasons in Egypt.

Infest fattening animals especially kept in stables with RH near 100%.

So, these species infest animals in lower Egypt.






18 20 days
5 - 6 days
larval stage
6 - 8 days
nymphal stage
7 - 12 days
adult stage
nymphs
moult
adults
moult
larvae
larvae climb
and attach
to beast
engorged female
drops
larvae hatch and
climb grass
eggs
female lays eggs
then dies

<u>B- Two hosts tick</u>

e.g. Hyalomma

"H. dormedarri"

"H. anatolicum"

"H. a. excavatum"

"H. rufipes"

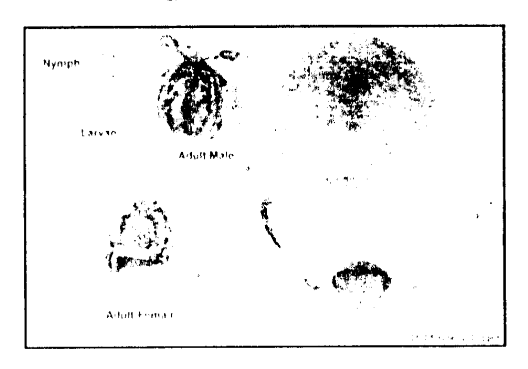
"H. schulzei"





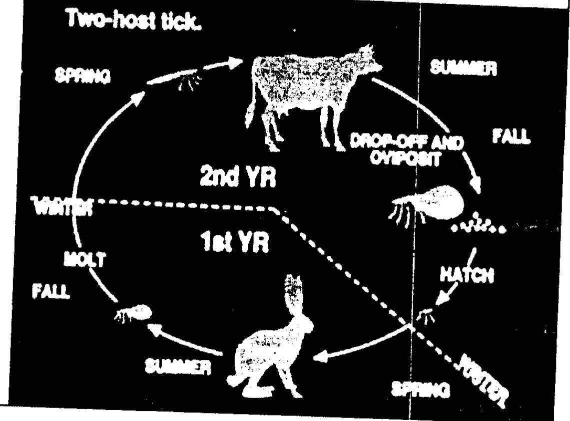
The ticks of this genus are the largest in size and mouth parts and have the largest legs.

Infect Camels, Buffaloes and Cattle in soft tissues araes (hairless reigons).



Infest animals in opened pasture of RH less than 80%.

These species need two hosts to copmlete life cycle.



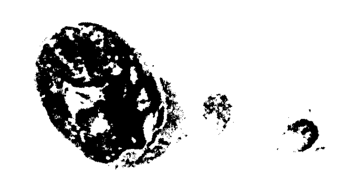
C- Three Host Ticks:

Rhipicephalus

"R.turanicus"

"R.S.sanguinis"

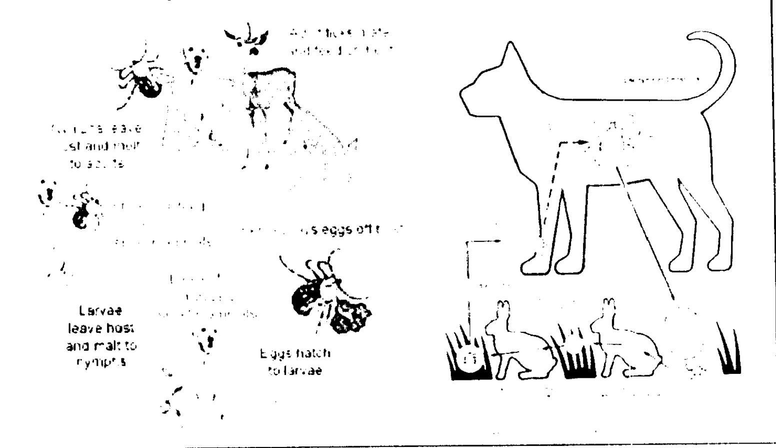




Infest Equines,Sheep,Goats,Rabbits,Dogs and Small Rodents.

These tick species need 3 host to coplete life cycle.

Infest ear,eyes,and between thigh.

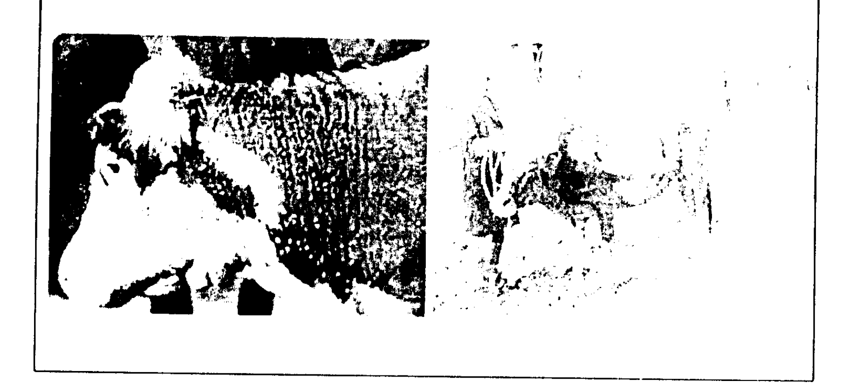


Economic Losses

Ticks are the most important ectoparasite of livestock in tropical and subtropical araes and responsible for sever economic losses both through the direct and indirect vectors of pathogens and toxins.

*** Feeding of large numbers of ticks cause reduction in live weight gain and anaemia.**

*Feeding of large numbers of ticks cause losses in production through diminished growth rate and decline in milk yield.



*Ticks bite reduce the quality of hides and depreciation in the value of hides contributes 5 % to the cost of tick problem.



*Tick infestation followed usually by abscess and myiasis due to secondary infection.



*Tick cause "otoacariasis" due to infestation of ear canal and blindness by affecting the cornea.



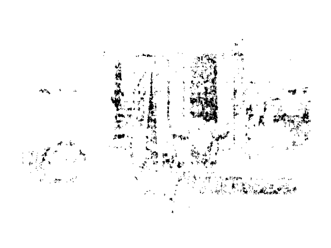
*Tick paralysis cause by the saliva in certain developmental stages in camel.
(Removal of ticks usually leads to immediate improvement).

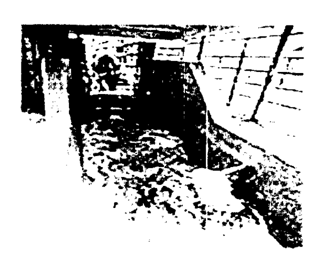




*Ticks are transmitters of harmful pathogens as virus,rickettsia,bacteria and protozoa.

*Losses due to control of ticks and tick-borne disease reached 12 billion U.S $.





The conventional method for control of ticks by the use of chemical acaricides had certain limitation such as:

*Resistance

*Enviromental Pollution

*Residues in meat and milk

*Higher cost that reach 10S/animal per yaer and the losses due to chemical control reached.

So,

The biological &immunological control of ticks is gaining importance and encouaging results which have been achieved in the past by immunizing various animals.

In recent years,developed antitick vaccines against "*Boophilus microplus*" based on tick antigen Bm&6 which is now commercially avilable in Australia (Tick GARD).

And similar vaccine developed and produced in Cuba (GAVAC).

Numerous investigators have used different parts like salivary glands and midgut as a vaccine immunogens which induced variable levels of protection.



BOVINE BABESIOSIS

BY

Dr. Magdy H. Al-Gaabary
Ass. Prof. Of Infectious Diseases.
Dept. Animal Medicine, Fac. Vet. Medicine,
Kafr El-Sheikh,
Tanta University

BOVINE BABESIOSIS
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Definition:

It is a tick - born disease of domesticated and wild Animals. The disease is caused by members of genus Babesia. The disease is characterized by fever, hemolytic anemia, hemoglobinuria, jaundice and frequently death.

Etiology:

Babesia are strictly intraerythrocytic parasites than the closely related Theileria, organisms of the two genera being collectively known as piroplasms. Some of the important Babesia species are listed in the table blew. They are described collectively as large or small species on the basis of their size (> 3 or< 3 um.) . Size is also a crude indicator of their susceptibility to chemotherapy; The larger the babesia the more the susceptibility to treatment.

The common Babesia spp. of domestic animals.

Host	*Babesia spp.*	*Large / small*
Cattle	*B.bigemina*	Larg
	B. bovis	Small
	B.major	Large
	B. divergens	Small
Equine	*B. caballi*	Large
	B. equi	Small
Sheep and goats	*B. motasi*	Large
	B. ovis	Small
Dogs	*B. canis*	Large
	B. gibsoni	Small
Cats	*B. felis*	Small

The Babesia spp. appear within RBCs. as non pigmented, pear shaped organisms which reproduce by binary fission. Two organisms are thereby commonly seen together, with there narrow ends in apposition. Amoeboid forms are also seen . Large spp. may be as long as the parasitized cell (6 um.) whereas small spp. may be only 1.5 um. In length .

Transmission:

In bovine babesiosis, B. bovis and B. bigemina are transmitted by one host ticks of the genus Boophilus, which are distributed world wide between latitudes 32 S. and 40 N., Transmission through this one host ticks is transovarial. Following detachment, the infected , engorged , adult female pass the Babesia to the larvae of the next generation through the eggs. Following the attachment to a new host, infection is transmitted by the larval and nymphal stages.

Pathogenesis and clinical signs :

The infective stage, sporozoite, invade the RBCs. where it multiply by binary fission, feed and become mature . Then it escape from that cell to invade a new one .These events lead to destruction of the parasitized RBCs. resulting in hemolytic anemia which is manifested by pale mucous membrane, accelerated respiration and heart rates. When the anemia become severe , anemic anoxia may developed causing death. The destruction of the infected RBCs. also leads to release of its hemoglobin, hemoglobinemia with subsequent prehepatic jaundice. When the hemoglobin exceeds the renal threshold, it will pass through the kidney showing bloody urine .The circulation of the protozoan with its metabolites results in fever which accompanied with congested mucous membrane, anorexia, rumenal stasis , cessation of lactation and depression.

In B. bovis , the infected RBCs. may lodged in the minute capillaries of brain leading to cerebral form of babesiosis which manifested by convulsions ,paddling and coma terminating by death.

PM. Changes:

Presence of ticks on the dead animal.

Enlarged and congested liver and spleen.

The gall bladder is enlarged and filled with dark thick bile.

Edematous and congested lungs.

The urinary bladder is filled with dark coffee-colored urine.

The kidney is congested.

Depending on the stage at which the animal die, the tissues may appeared congested, anemic or icteric

Diagnosis:

Field diagnosis:

Fever, anemia, jaundice and hemoglobinuria are suggestive clinical signs of babesiosis in cattle located in enzootic areas where *Boophilus* ticks occur.

The disease should be differentiated from; hypophosphatemia, leptospirosis and bacillary hemoglobinuria.

Lab. Diagnosis:

Samples:

Thin blood film.

Thick blood film.

PM. Impression smear.

These Samples should be fixed, stained and examined microscopically for detection of the organism.

Treatment:

Dimenazene diaceturate ;Berenil 3-5 mg. /kg.

Imidocarb. 1-3 mg./kg.

Control:

Treatment of the infected animal.

Chemo prophylaxis.

Tick control

Vaccination depending on the phenomenon of premunition and endemic stability.

THEILERIOSIS

It is the infection with the *Theileria spp.* which characterized by fever, lymphadenopathy and anemia.

Etiology and Spp. affected :

Theileria parva complex ;

T p parva causing East cost fever

T p bovis causing Jan. disease.

T. p. lawerenci causing corridor disease

These diseases affect cattle , transmitted by *Rhipicephilus spp.* and distributed in eastern and central parts of Africa.

Theileria annulata; causing Bovine tropical theileriosis, Egyptian fever or Mid. Cost fever.

The disease affect cattle, transmitted by *Hyalomma spp.* and distributed in north Africa, middle east, south Europe Indian subcontinent and China.

Theileria mutans ; causing Benign bovine theileriosis, transmitted by *Amblyomma spp.* with world- wide distribution.

Theileria sergenti causing Oriental theileriosis, transmitted by *Hemophysalis spp.* confined to Japan.

Theileria hirici; causing malignant ovine theileriosis ,transmitted by *Hyalomma spp.* and distributed in North Africa and Middle East.

Theileria ovis ; causing Benign ovine theileriosis, transmitted by *Rhipicephilus spp.* with world wide distribution.

Transmission:

Transmission of infection occur by ticks by stage to stage transmission . The erythrocytic stages were contracted by the larval or nymphal stage of ticks in which , it develop to be sporozoites, the infective stage, in the salivary glands of the second stage, nymph or adult respectively.

Pathogenesis and clinical signs:

The nymph or adult stages inject the sporozoite in the host animal. The sporozoite then invade the lymphocytes which is greatly sensitized by such invasion resulting in high rate of proliferation. In the lymphocytes the invading sporozoite will multiply by schizogony leading to macroschizonts and microschizonts . The lymphocytes then ruptured releasing its contents of merozoites which can invade the erythrocytes with limited simple binary fission resulting in 2- 4 erythrocytic stages . Such stages are infective for ticks.

The clinical signs may summarized in the following:

Fever which last 7 - 10 days.

Rumenal stasis

Loss of appetite

Severe reduction of milk production.

Enlargement of the superficial lymph nodes

The mucous membrane is congested at first then become anemic

Signs of pulmonary edema .
Corneal opacity
The erythrophagocytosis leads to anemia that appeared as:
Pale mucous membrane
Accelerated heart rate
Accelerated respiratory rate
At the early stage , constipation is evident
During the late stage of the disease bloody diarrhea may occur.

PM. Changes:

Presence of ticks on the dead animal
Enlarged liver and spleen
Edema in the lungs
Enlarged lymph nodes
Anemic tissues
Ulceration of the abomasal mucous membrane
Peticheial hemorrhage in the epicardium and endocardium

Diagnosis:

Clinical findings
Thin blood film
Lymph node smear.
Liver biopsy
PM. Impression smear
IFA.

Treatment:

Oxytetracycline 10 mg. /kg.
Arrhinal 10 %
Buparvaquone 2.5 mg. / kg.
Parvaquone 10 mg. / kg.
Halofuginone lactate 1.2 mg. / kg.

Control:

Tick control
Treatment of infected animal
Trials of vaccination

MICROFILARIA (MF)

By Prof. Dr. Taher Mohamed Abdel Wahab

Director Of Kafr El Sheikh Regional Animal Health Institute

Head Researcher Of Parasitology

MICROFILARIA (MF)

It is the embryo or the pre larval stage of the filariid worm.Since the sexually mature female filariid worm release agreet numbers (swarms)of microfilariae (MFs),these microfilariae migrate to or usually present in the peripheral blood, lymph stream or in the superficial layer of the skin, and they need a vector (arthropod intermediate host) in which they differentiates into a fully infective third stage larvae(L3)which transmitted to anew host during the feeding of this vector on this new host.Fig.1.

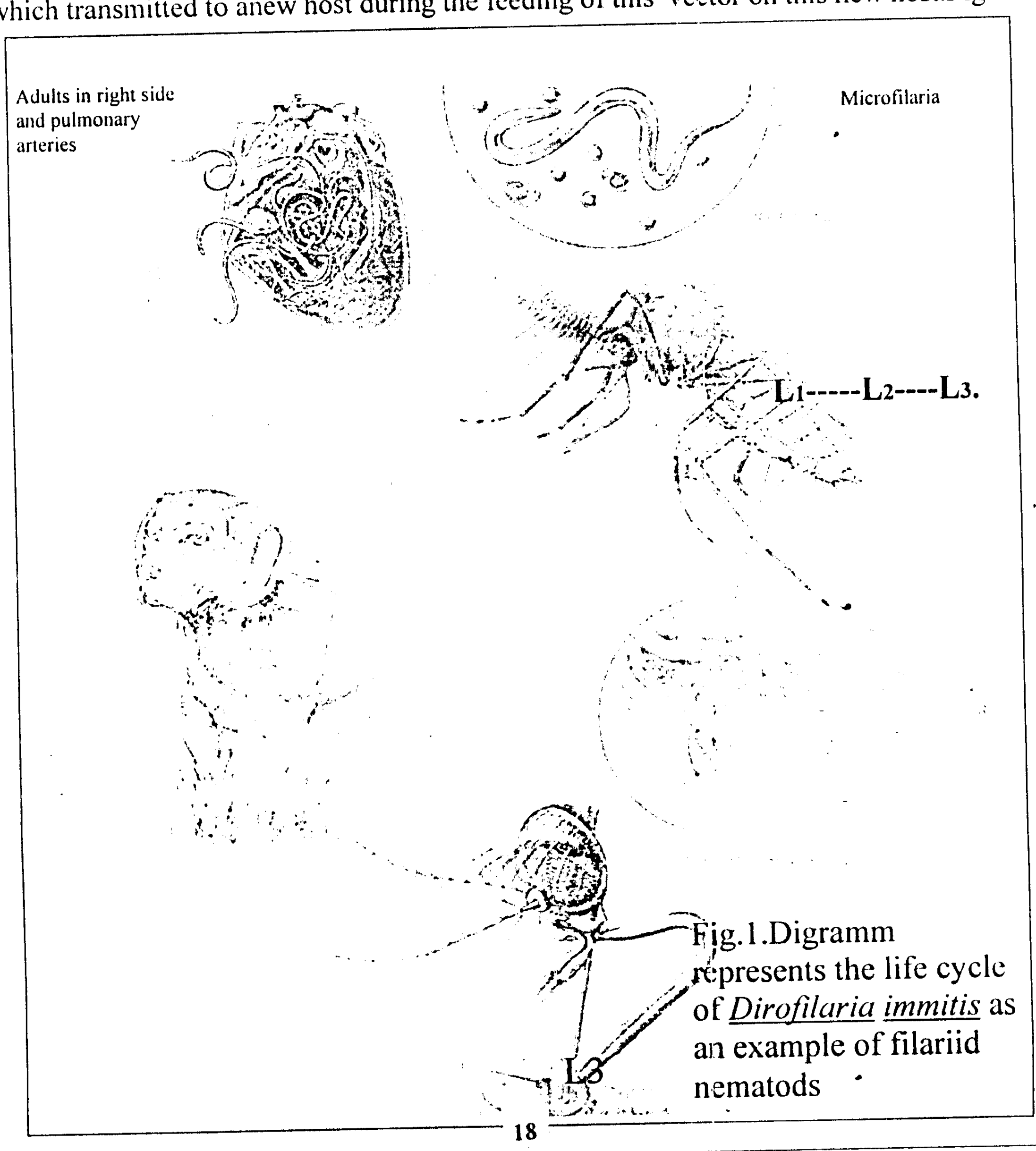


Fig.1.Digramm represents the life cycle of *Dirofilaria* *immitis* as an example of filariid nematods

CHARACTERS OF MICROFILARIAE:

1- Microfilariae were released in thousands or millions and become active at or shortly before birth; they do not exhibit the normal anatomy of nematode larvae ;they are undifferentiated having no; oesophagus; no elementary tract or any structure (just only a column of nuclei) .

2- The size of the microfilariae varies so greatly and can not be used for differentiation.

3- They are elongated, slender and may be coiled or slightly straight, with rounded or blunt anterior end. Certain species have graceful flooring curves, others are irregular. The caudal end may be curved (*Onchocerca cervicals)*, straight(*Dirofilaria immitis)*, pointed or rounded.

4-In fresh smear microfilariail motility may differ from one species to another;for example micfilaria of *Dirofilaria repens* undulate in its place;microfilaria of *Dirofilaria immitis* ***moves in ajerky erratic fashion backlashing on themselves with very little forward movement while those of Diptalonema reconditum showed amore rapid regular forward movement that tended to take them out of the microscopic field of vision.***

5-The cuticule may be smooth or transversally striated.

6- Abcence or presence of the microfilarial sheath is distinguishing feature. In some species the egg membrane ruptures liberating an unsheathed MFs; in other it forms aclose-fitting elastic sheath, also it may be loose and extend beyond the extremities of the body. It may be oval and not permit the MF. To stretch itself [such forms are generally found in the skin of the host and may not be able to move in capillaries and lymphatics (not all MFs in the skin are of this type)].

7- Microfilaria appeared to be filled with minute oval or round shaped nuclei(primordial cells) which occupy the entire width of its body. The arrangement of these nuclei is of much important for the survival of MFs as it facilitate its passage through the fine capillaries and through the narrow mouth part and gut wall of the arthropod vectors. The percentile (%) location of certain anatomical structures differs in different species,of filariid nematod these anatomical structures are called (LANDMARKES)

LANDMARKS

Are interruptions in the nuclei,of the microfilaria or deeply or faintly stained nucleated cells these landmarks are;(1)cephalic space (2)nerve ring (3)ecretory pore and excretory cell. (4) four genital cells(deeply stained cells) 1st, 2nd,3rd,and 4th genital cell. The location of the 1st genital cell and its relative position to the 2nd,3rd and 4th genital cells is important in differentiation between different species of filariid microfilaria.

Since different methods of fixation causes various degree of contraction of the MFs, it is customary to express the distance of certain fixed points from the anterior end as percentages of the total length (in this way accurate description of the MFs is obtained), also the position and arrangement of the terminal nuclei is important in differentiation between different species.of filariid microfilariae (fig.2. 3. 4. 5. and table 1. , 2)

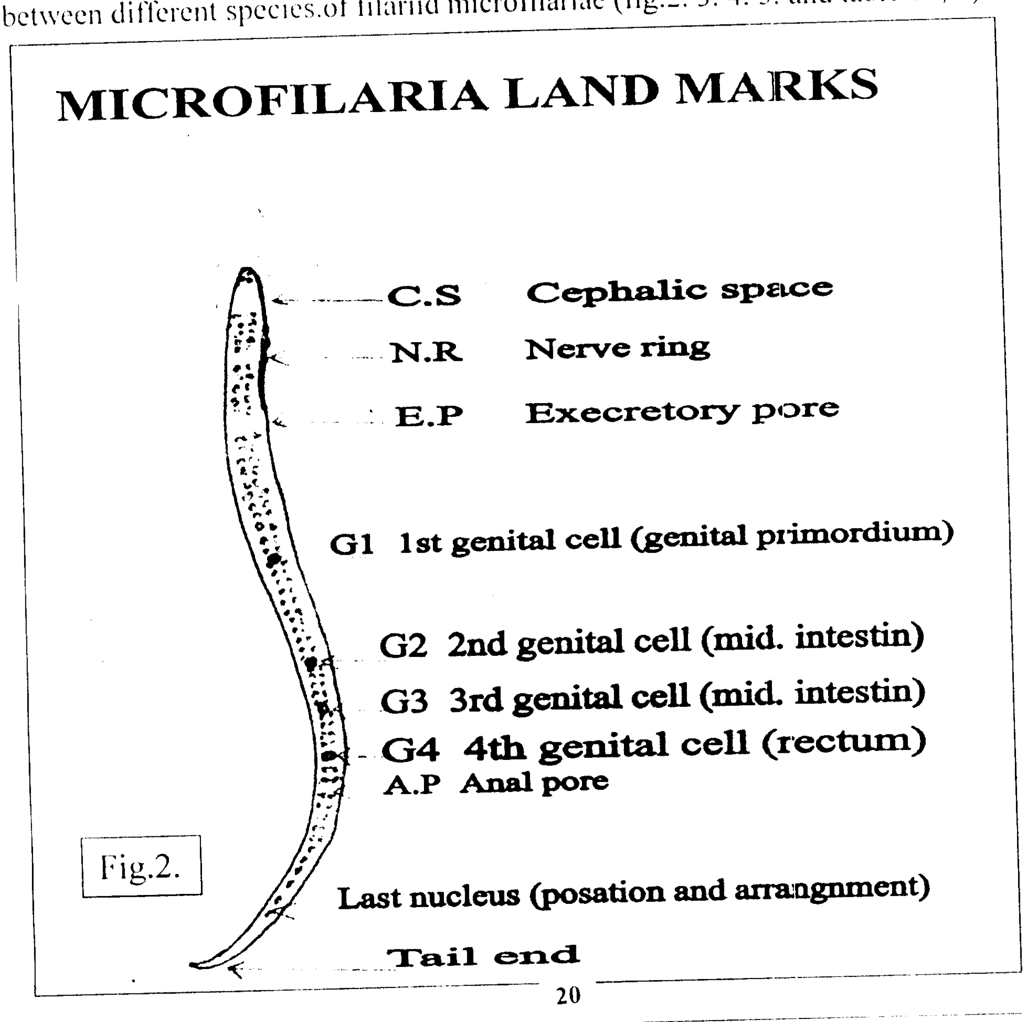


Fig.2.

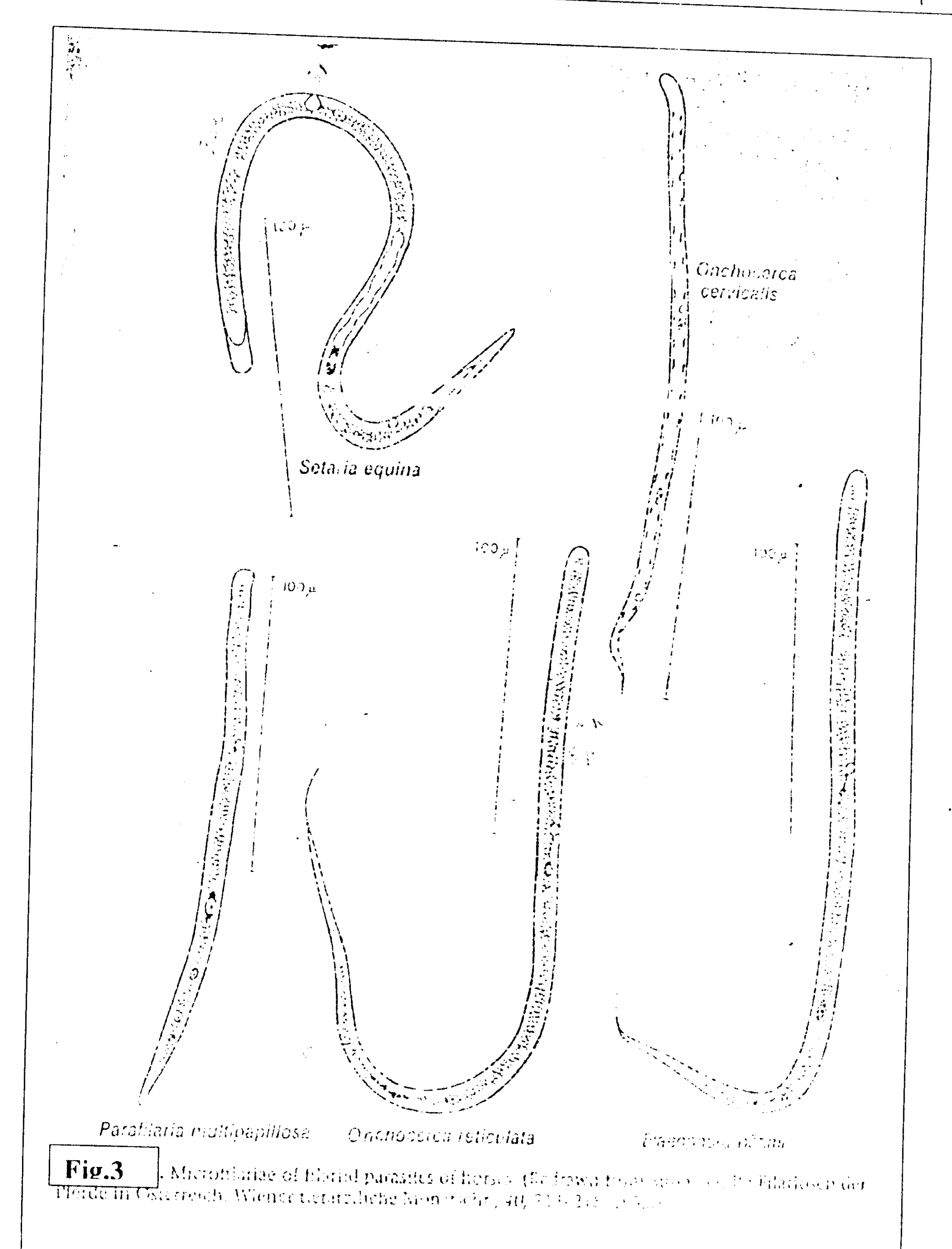


Fig.3. Microfilariae of filarial parasites of horses [illegible] Filariosen der Pferde in Österreich. Wiener tierärztliche Mon[illegible] [illegible]

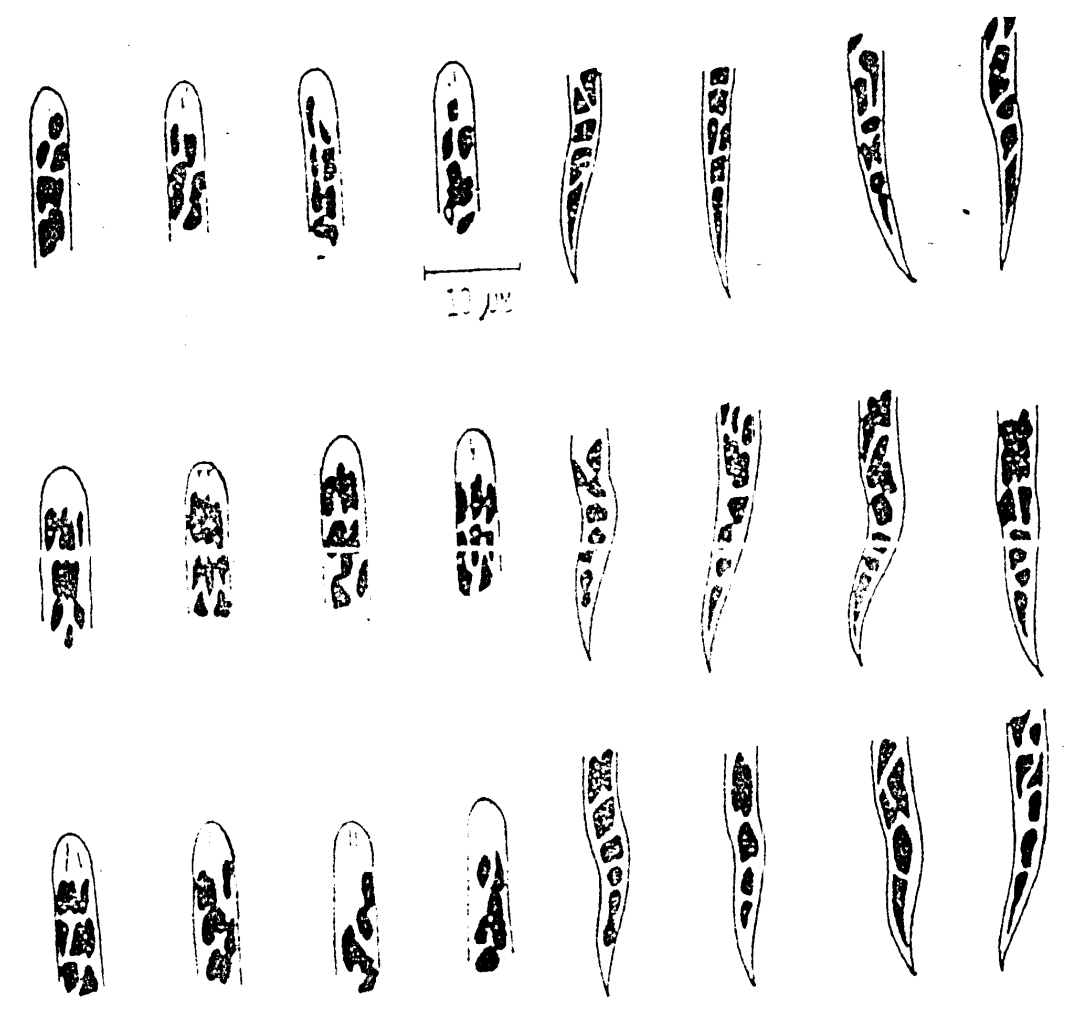


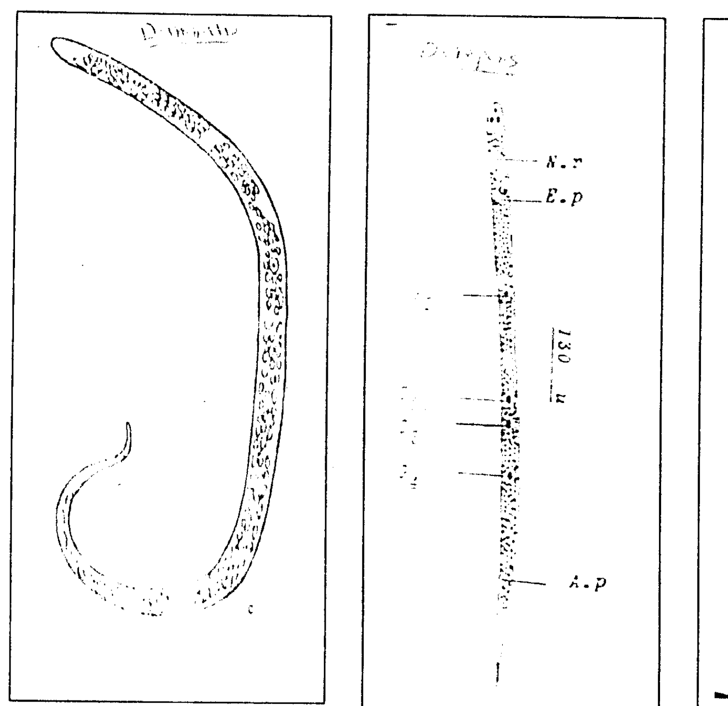
Fig.4. Arrangement of cephalic and caudal nuclei in *Onchocerca* spp. microfilariae.
O. cervicalis (top row). *O. gutturosa* (middle row). *O. volvulus* (bottom row).

Table: (1) **Microfilariae of filariid parasites of horses.**

Aspects	Setaria Equina	Onchocerca cervicalis	Onchocerca reticulata	Parafilaria multipapillosa
• Length (um)	223-242.4	207-240	330-370	122-190(156)
Width (um)	4.8-6.7	4-5	6-7	3.6-5.6(4.3)
Location of constant point in percent to total body length.				
Cephalic end - nerve ring	4.78	23.78	17.76	17.05
Cephalic end-- excretory pore	24.3	42.71	27.6	24.68
Cephalic end -- G_1. cell	32.43	68.08	59.17	55.1
Cephalic end –anal pore	78.4	80.58	72.80	84.55
sheath	Sheathed Delicate transparent sheath; longer than the MF.; protruded at both extremities	Non theathed; The post end is narrow and curved ;the tail is bent side way	nonsheathed	nonsheathed

Table: (2) Some Microfilariae of dogs

	Dirofilaria.immitis.	Dirofilaria.repens.	Dipetalonema.recondatum.
Fresh smear:	Few to many in number.	Few to many in number	Always few in number.
	Jerky erratic fashion back lashings on themselves without aim.	Undulate in its place.	Rapid movement across the field that tend to take them out of the field of vision..
Shape in stained smear:	Curved.	Appear straight.	cresentic in shape.
Anterior end:	Rounded	Tapered	Blunt
Tail:	Straight,sharplly pointed tail,rarely deviated from the long axes.	Straight tail.	Have a button hooked tail.
Length:	313(u.).	270-317(u.).	230-279(u.).
Width:	6.9(u.).	6.3-7.2(u.).	2.3-4.3(u.).
		The column of nuclei does not reach the end of the tail.	



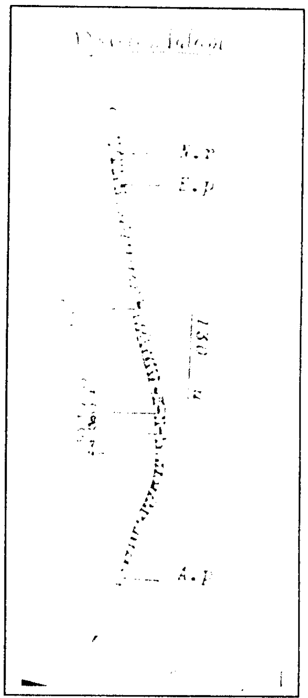


Fig. 5

Vector

Microfilaria needs an arthropod intermediate host which may be: -

- Blood sucking [mosquitoes, lice, fleas and hard tick].
- Some species are transmitted by musca.

During feeding of the vector the microfilarae are taken by the proboscis and develop in the body cavity to infective third larval stage L_3; which again reach the vertebrate host during other blood meal . this third larval stage reaches to specific tissue where they mature and mate .(fig.6)

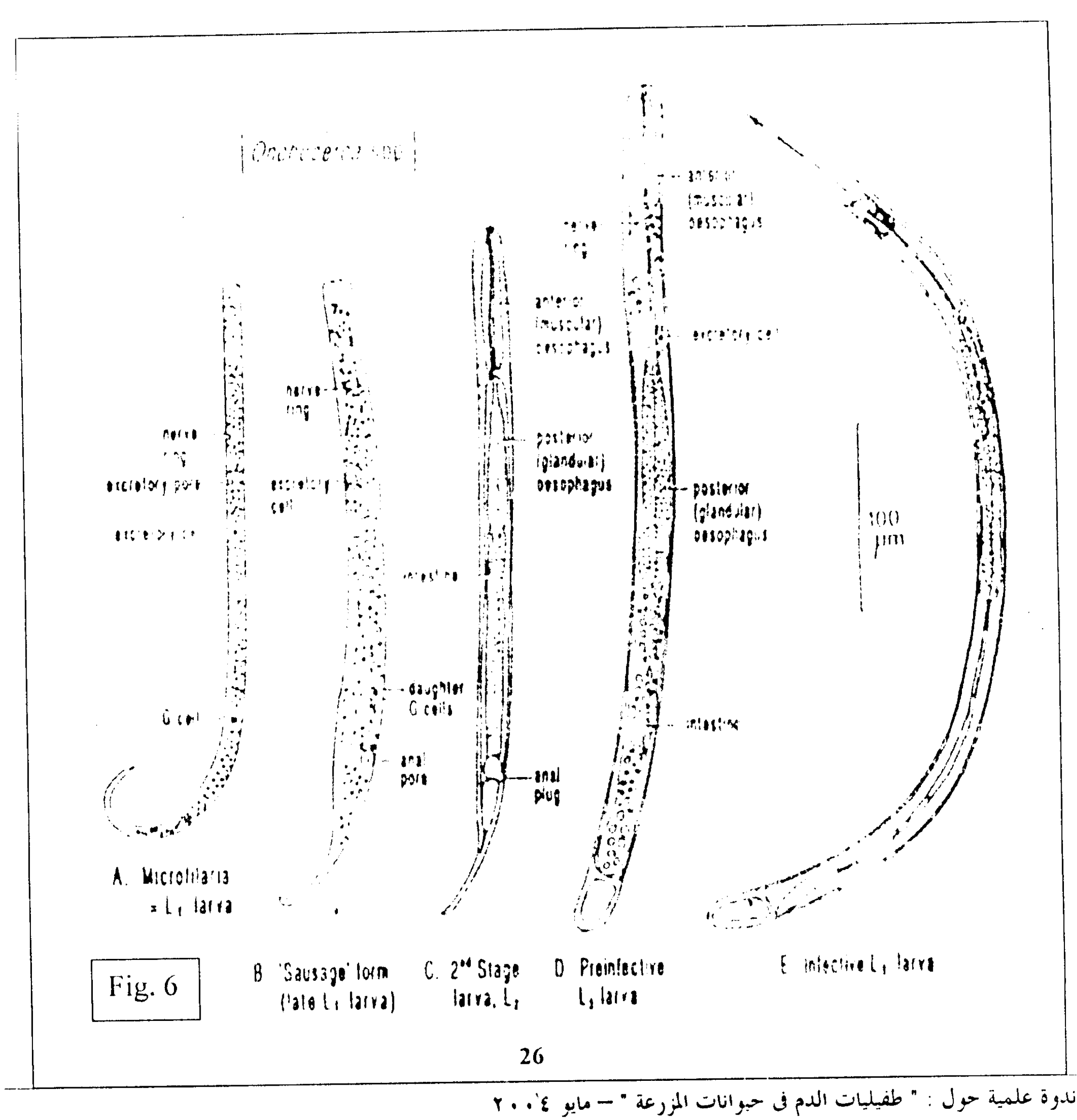


Fig. 6

EXISTANCE IN THE HOST:

1- Microfilaria of *Dirofilaria. immites* (heart worm of dog) appears in about 8 month in the blood of dog after exposure of the dog to infection, while (MFs) of *Wucherria. bancrofti* appears in 3 years after exposure.

2- Microfilaria may survive in the host for several weeks either after the death or destruction of the adult worms or if they transferred to uninfected animals ; for example MFs .of *Litmosoide.carinii* remain in the blood of cotton rat 2 month and those of *W. bancrofti* in blood of man for 4-5 month while those of *D. repens* live more than 2 years in transfused dog .

3- Microfilaria of *D.immitis* pass through the lympth nodes of experimentally infected dogs without phagocytic destruction while those of frogs and dove are filtered out the circulation by the liver and spleen and eventually destroyed by reticuloendothelial cells.

4- Microfilaria are released from adults in thousand or million and when died turn over occur again .

5- MFs of *D .immitis* survive in vitro 10 days and in refrigerator for 3 weeks.

Correlation between host and parasite and vector

Periodicity

The microfilaria (MFs) of certain species of filariid nematod either sheathed or un sheathed appeared in the peripheral blood stream of the final host either durung the night (nocurnal periodicity) for example *(W. bancrofti)* vectored by mosquitoes or during day(durinal periodicity) Example *(loa loa)* vectored by tabanid fly (chrysops).

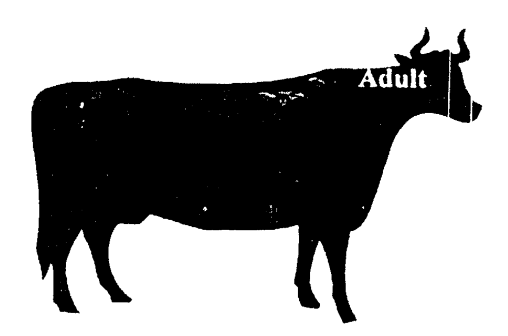
Hawking (1964) during its work on *W.bancrofti* indicated that periodicity is a character of MFs and not the host he found that MFS accumulate in the lung during the day by afixative force.this fixative force is increased by raising oxygen tension in the pulmonary capillaries by giving O_2 or administration of isopronalin. He cited that MFS responded to physiological changes in the host and flood in the blood during night Harold townson (1977) cited that a nocturnally periodic *W. bancrofti* become diurnally periodic if the individual sleeps by day.

Hawking (1967) points out that the behavior of MFS appears to be adapted to promote transmission by arranging the maximum number of MFS in the peripheral blood at times when the arthropod vector is likely to bite.

Microfilariae of *Onchocerca gutturosa* concentration in the umbilical region of the British cattle which is consistent with their transmission by *simulum ornatum* which preferentially feed in the midline area, while in tanaznia ,MFS of *O. gutturosa* were conc. In cephalic region (ear) this was consistent with simulium vorax which feed primarily on the ear (fig.7).

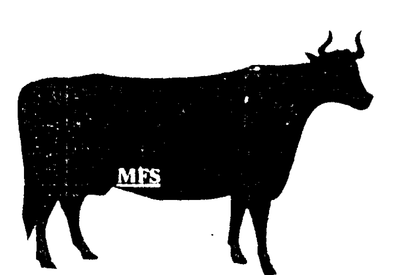
Microfilariae of O.cervicals migrate from the region of the weither and the ligamentum nuchae through the subcutis to localize in the dermis;to all partes of the body specially in the ventral abdominal midline (umbilical region showed the higest concentration).This distrepuation coinsided with the biting of midges of the genus Culicoides and mosquitos of the genus Anopheles;but thefull vector range is not known. (Fig.8)

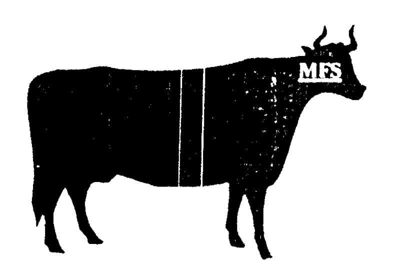
Onchocerca gutturosa



=British cattle
-Simulum ornatum

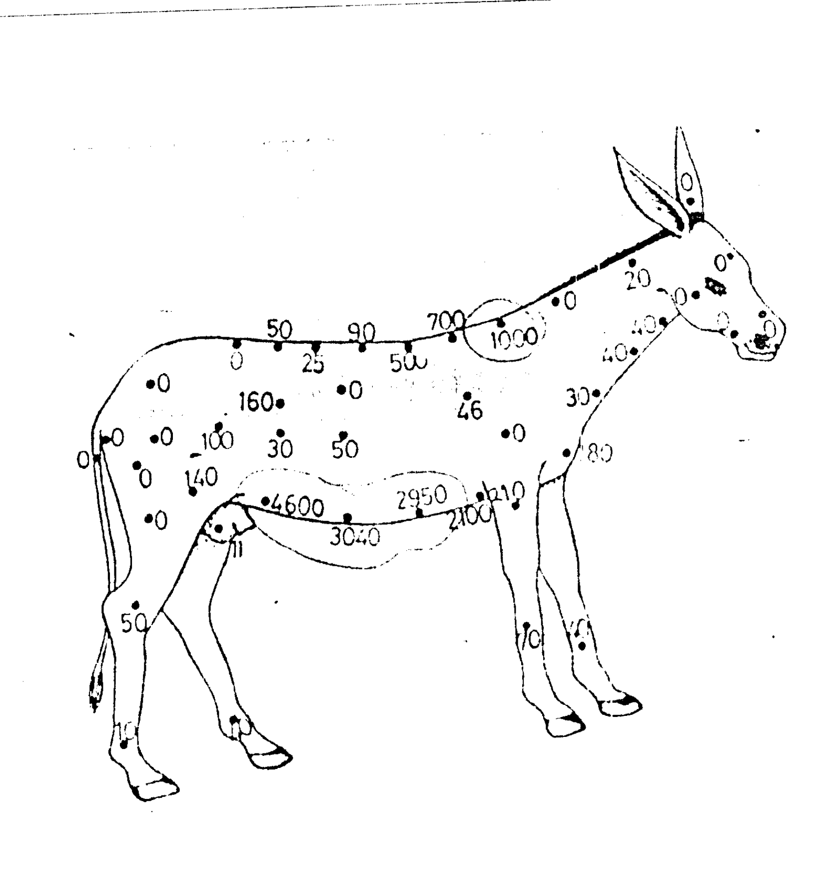
=Tanzinia
-Simulum vorax





* There is a directional mechanism which insures the migration of the MFs, perhaps this stimulus is in the form of chemical gradient, or combination of factors which have developed over along association between parasite and host

Fig.7



-Distribution of Microfilariae in the epidermal layer of skin.

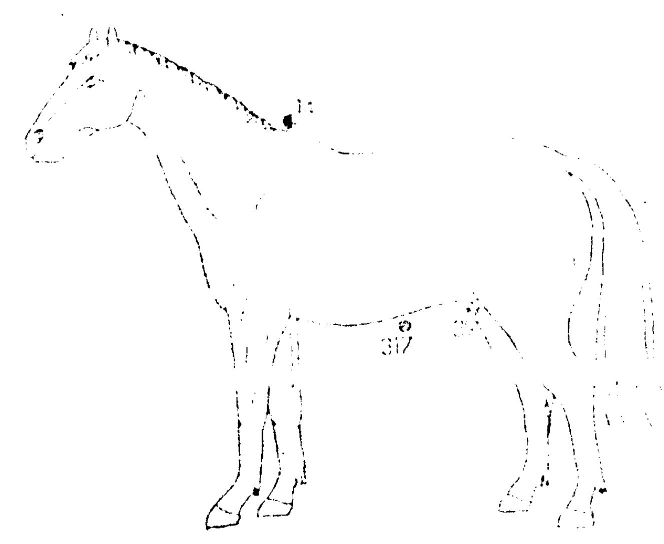


Fig8.

-Landing and biting sites of Culicoides nubeculosus

Pathogenesis of microfilarae (MFS).

Adult female worms produce a more or less steady supply of living embryos mounting to many millions during each female life time, these embryos invade the peripheral blood stream, lymph stream or superficial layer of skin, they also invade the delicate tissues of the eye as well as the lymph nodes and some deeper internal organs .there is a continuous turnover of microfilaria(MFS) resulting from their death in the tissues. In endemic areas with high rates of transmission comes heavy concentration of (MFS) build up in the skin.

The presence and the death of thousands or millions of microfilaria in the skin of in leads to severe irritation and dermatitis followed by atrophic and pigmentary changes in the skin.

(1)-Setaria:-

(a)-Microfilaria of setaria species may penetrate the anterior chamber and chornia of the eye causing blindness or impaired sight as the result of traumatization of the eye by living microfilaria or due to the inflammatory immune response to micrfilarial antigen or dead microfilaria .

(b)-microfilaria of setaridae may migrate through the central nervous system causing neurological disturbances which vary from muscular weakness to ataxia and paralysis.

(2)-Stephanofilaria:-

(1)-adult worms are found in the dermis, while its microfilarae occupy a more superficial position in the dermis causing hairless irritated area around the eye. The adult worm with its MFS cause skin inflammation and destruction of the hair follicles and skin gland causing raised areas of papules from which blood may ooze. Later on these papules join to make large lesions which may reach (25 cm.) in diameter .the lesion can be seen on the udder on the ventral surface of the abdomen.the animal tends to rub the lesion making them worse.

(3)-Dipetalonama (in camels):

(a)-micrifilaria with the adult may cause orchitis and testes enlargement in camels, arteriosclerosis and heart insufficiency.

(b)-canine dipetalonema must be differentiated from those of *D.immitis* in diagnosing heartworm infections.

(c)-*Dipetalonema streptocerca* (in man) Live as adults and MFS in skin and may cause (minor elephantiasis).

(4)-Onchocercidae:

(a)- Microfilarae of *Onchocerca cervicalis* migrate through the skin and s/c tissues to localize in the dermis specially the ventral abdominal midline ,inguinal and perineal areas . Hypersensitivity reaction to microfilarial antigens manifested by:

- Pruritic skin reaction known as summer mange, or equine dhobi or kosen .
- Typical onchocercal annular lesions on horses head showning alopecia,
- Scaling and crusting of the superficial layer of skin.
- Equine periodic ophthalmia as well as keratitis .

(b)- Micrifilariae of *Onchocerca reticulata* cause chronic dermatitis in solipeds.

(c)-Adult filaria as well as its microfilaria of *onchocerca reticulata* and *onchocerca cervicalis* play a role in causing fistulous weither.

(d)-Reaction to dead microfilaria in the skin can lead to several unpleasant condition (destruction of the elastic tissue and formation of redundant folds in the skin {*Onchocerca volvulus*}).

(5)-Dirofilaria:

(a)-great attention was paid to the heart worm of dog *Dirofilaria immitis* and to *Dirofilaria repens* as a zoonotic diseases transmitted from dogs to man producing a serious lesions either in the skin or internal organs ending with carcenoma of the lung in some cases.(*Dirofilaria immitis* do not mature in humans but the larval stage has been reported in cutaneous nodules as well as in the lung producing lesions in the lung) .

(b)-In dogs microfilariae of *Dirofilaria immitis* cause pustular and follicular eruptions on the limbs and back of the dog .

(c)-Microfilariae of *Dirofilaria repens* cause eczematous dermatitis ,intense irritation and ulceration of the skin of limbs and back of dogs .

DIAGNOSIS OF FILARIASIS

• CLINICAL DIAGNOSIS :

Depends on a history of exposure to infection an endemic area and on the clinical manifestations.

• LABORATORY DIAGNOSIS :

Parasitological Diagnosis.

– Microfilariae :

Techincal skill of the examiner in differentiating between species of microfilariae specially in areas where animal filariasis co-exist with human filariasis.



a- Wet mount.
b- Thin or thick stained blood films.
c- Concentration method (Fulleborn technique).
d- Skin biopsy from the site of predilection of the MFs is teased apart and suspended in warm normal saline and incubated for at least 6 hours if the animal was infected MFs will be in the deposit.
e- The epiderms may be shaved off a skin site and the fluid expressed examined for MFs.
f- Histochemical methods for acid phosphatase has also proved of value.
g- Isoenzyme analysis is of value in some species.
h- Chromosomes examination.(*O volvulus* And *O gutturosa* can readily be distinguished)
i- DNA probes has now been developed for species of *Brugia*;*Wucherria*;*Onchocerca*;*Loa loa*.

Adult :

a- Microscopical examination to the collected worms
b- Histopathology for the suspected tissues (biobsy)

- Serological diagnosis :

a- Complement fixation test.

b- Intradermal test (with Dirofilaria antigen).

c- Mazzotte test:

by giving the suspected case 50 mg of diethylcarbamazine

→ pruritic rashes with 24 hours if the animal is positive.

d- Circulating filarial antigen:

a rapid format immunochromatographic card kit test (ICT) is easy and fast to perform in the field in a few minutes.

e- ELIZA. hihly sensitive test.

CONTROL OF FILARIASIS

Currently there are no vaccinations available to prevent Filarial infection so, control of Filariasis depends mainly on 3 main measures.

1- Vector control.
2- Treatment of the reservoir.
3- Surgical treatment specially in case of Onchocercal nodules.

1- Vector control:-

It is very difficult to control blood sucking insects [but only trials to reduce vector populations].

a- Chemical control:-

Spray or dusting or pour-ons or ear tags [alternating tags of different chemicals each year] this requires strategy that will give maximum control with the least number of treatment to reduce the risk of resistance as well as the risk of chemical residue levels in the environment (meat and milk)

b- Non chemical control:-

Fly trap and Natural enemies.

2- Treatment

There are Two types of treatment

a- Macrofilaricides [drugs directed against adult worms]

b- Microfilaricides [drugs directed against microfilariae]

Drug treatment will be effective if given before the disease become advanced.

a- Macrofilaricides

a-1. Suramin. [Antrypol]

- **It was introduced in 1920 for the treatment of African trypanosomiasis**
- **It has alethel action on adult *W. bancrofti* and other filarial worms but its toxicity and the availability of other filaricides reduced its use also it must be given intravenously**

a-2. Moxidectin

- **Have interesting Macrofilaricides activity in animal models of Onchocerciasis; Lymphatic filariasis and others and is now ready for evaluation in human**
- **Singal dose kills or sterilize the adult worms.**
- **Half life of this drug in animals is approximately 10 times that of ivermectin; thereby reducing the probability of successful reinfection.**

a-3. Diethylcarbamazine (Piprazine derivatives) [DEC)

- **6 mg/kg Bw**
- **Potent microfilaricide**
- **Have limited efficacy against adult**
- **It was registered for use in lymphatic filariasis, it used for pet animals.**

Disadvantage :

- **Some patient experiences severe systematic reaction specially in heavily infested one (as it is potent microflaricide by sensitizing the MFs to phagocytosis).**
- **N.B. no toxic effect on uninfected individuals.**
- **This adverse reaction can be minimized by spreading the treatment in weekly low doses or putting it with the table salt.**

DEC-fortified salt

- **Common salt can be fortified easily and cheaply with DEC.**
- **The drug is very stable survive cooking and is tasteless.**
- **Small amount taken over a period of months to years can eliminate microfilariae and interrupt transmission completely.**

a-4. Albendazole : Macroilaricide

Parasitology today vol. 15, No. 9, 1999.

- **As a single dose with DEC or ivermectin blocks the enzymatic process responsible for absorption of glucose by the parasites, causing its death.**

a-5. Metrifonate : (Trichlorophan).

- **Organophosphorus insecticide effective in treating nematode infection it acts by inhibiting cholinesterases and thus paralysis the worms [Toxic if increased than 10 mg/kg B.w.].**

b- Microflorrcidal drugs

b-1. Ivermectin

- **Only microfilaricide for most filarial infection.**
- **It activate (GABA) pathway.**

Advantage:

- **Single dose administration**
- **It acts more slowly than DEC producing less effect than DEC [so the acute reactions associated with DEC can `be avoided] and only pruritis is the most common side effect. So**
- **Oxford textbook of medicine recommended that the patient be treated with ivermectin at the dosage 50 mg/kg. Bw to eliminate *O. volvulus* MFs before DEC therapy is initiated for treatment of loaiasis.**

Resistance :

Resistance to ivermectin might developed and spread.

- **Life spane of adult worms more than 10 years in man and repeated use of this drug seem to have some permanent effect on the fertility of the worm. [this was agree with our result, that i.v. paralysis the MFs inside the uterus.** Abd El-Wahab *et al.* 2002.

b-2. Mebendazole :

- **Potent antithelmentic with wide range of activity against nematodes, cestodes and both tissue stages of the parasite as well as against worms in the lumen and gut.**
- **As the drug is poorly absorbed high doses must be used when treating tissue infection (filarial infections)**
- **It stops the development of embryo in Onchocerca and when given with levamezol, both microfilaricidal and embryostatic effects can be achieved. MFs counts in the blood fall slowely and the effect may be last for 6 month.**

a-3. Levamazol :

acts by interfering with the carbohydrate metabolism of the nematodes.

Trypanosomes of farm animals

By
Dr. Abdel-Razek Y. Desouky,
DVM, Ph.D.,
Ass. Prof. Of Parasitology
Faculty of Veterinary Medicine,
Kafr EL-Sheikh, Tanta University

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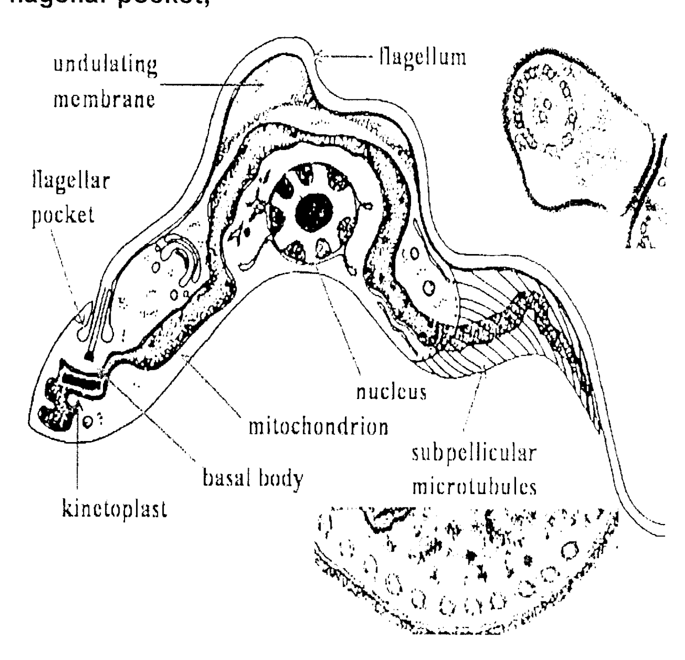
Ass. Prof. Of Parasitology

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Phylum Euglenozoa
Subphylum Kinetoplasta
Class Trypanosomatidea
Order Trypanosomatida
Family Trypanosomatidae
Genera: Trypanosoma, Leishmania

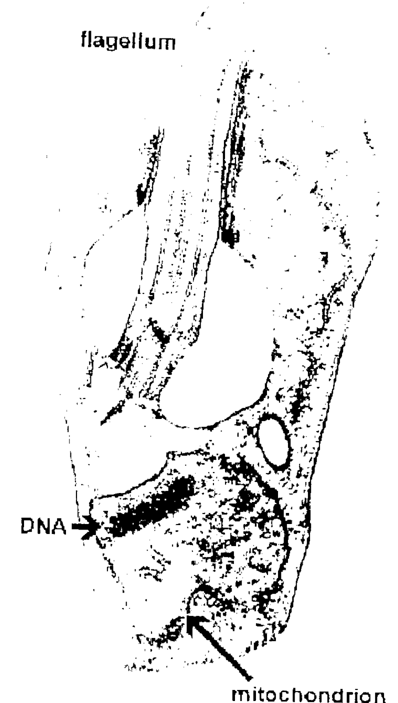
kinetoplast
nucleus

Phylum Euglenozoa
Posses cortical microtubules, flagella usually has a paraxial (crystalline) rod - a rod that runs along side the axoneme in the flagellum.
Subphylum Kinetoplasta
Possess a unique mitochondrion with a large disc of DNA, made of both mini- and maxicircles.
Class Trypanosomatidea
One or two flagella arising from a flagellar pocket; single, tubular mitochondrion extending the length of the cell; usually possess a single DNA containing kinetoplast near the flagellar kinetosomes.
Order Trypanosomatida
Single flagellum either free or attached to the cell via an undulating membrane
Kinetoplastida
Members of this group are characterized by the presence of a kinetoplast, a spherical, rod- or disc-shaped structure; probable serves to provide energy to the kinetosome, a modified mitochondrion, and is made up of linear molecules, minicircles and maxicircles. The circular molecules (minicircles and maxicircles) contain DNA (kDNA) and it undoubtedly codes for structures and functions related to the mitochondrion. The kinetoplast lies just posterior to the kinetosome; the structure from which the flagellum originates
The kinetosome has the classic centriole construction (9+2 fibril arrangement).



Family Trypanosomatidae

Species within the group have a single nucleus and are either elongated with an protruding flagellum or more rounded with a nonprotruding flagellum.



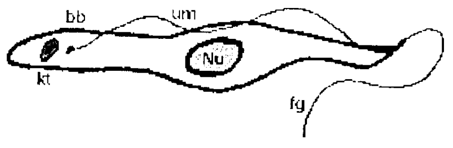
Those parasitic in vertebrates generally require an intermediate host (usually a blood sucking insect); the vertebrate parasites would be referred to as heteroxenous - occurs with more than one host during its life cycle.

Those parasitic with invertebrates can undergo their entire life cycle within the same host; referred to as monoxenous, since they have only a single host during the entire life cycle.

Among the trypanosomatids there are 8 main body types or morphs. They vary in size, body shape, location of the flagellum, location of the kinetoplast, and other structures .

Kinetoplastid Morphology

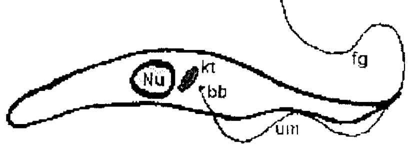
Several different morphological forms of kinetoplastids are observed. These various morphological forms are associated with different life cycle stages in the various species. The different forms are distinguished by the position of the kinetoplastid in relation to the nucleus and the presence or absence of an undulating membrane. The four major morphological forms found in kinetoplastids which cause human disease are:



trypomastigote

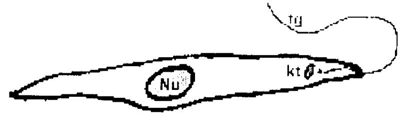
The kinetoplastid (kt) is located posterior to the nucleus (Nu), usually on the most posterior end of the parasite. The flagellum emerges from the posterior end and folds back along the parasite's body. This attachment of the flagellum to the body forms an undulating membrane (um) that spans the entire length of the parasite and the free flagellum emerges from the anterior end. The undulating membrane functions like a fin and increases the motility of the organism.

epimastigote



The kinetoplastid (kt) is more centrally located, usually just anterior to nucleus (Nu). The flagellum (fg) emerges from the middle of the parasite and forms a shorter undulating membrane (um) than observed in trypomastigotes. Epimastigotes are noticeably less motile than trypomastigotes.

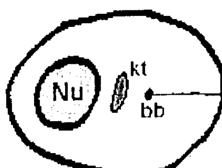
promastigote



The kinetoplastid (kt) is towards the anterior end and a free flagellum (fg) with no undulating membrane emerges. The end that the free

flagellum emerges from in all three motile forms is designated as the anterior end because they swim in that direction. In other words, the flagellum pulls the organism.

amastigote



The parasite is more spherical in shape and has no free flagellum. The kinetoplast (kt) is usually detectable as a darkly staining body near the nucleus (Nu). This form is a non-motile intracellular stage.

Genus Trypanosoma

The disease produced by such forms is termed trypanosomiasis" One curious feature of some pathogenic species is that they may also be parasitic in other animals in which they are apparently harmless In these natural hosts, the tissues have presumably become physiologically adapted to the presence of the parasites through long periods of association Consequently, such animals act as "reservoir" hosts (animals that harbor an infection that can be transmitted to humans) for these potentially pathogenic species. For most trypanosomes, the life cycle includes both an invertebrate and a vertebrate host. They are usually found in the body fluids of vertebrates, especially the blood and tissue fluids. But some forms (e.g. T. cruzi) occur intracellularly.
They typically occur in the digestive tracts of invertebrates, especially arthropods. Trypanosomes are divided into 2 main groups or "sections": Salivaria and Stercoraria. The distinctions are primarily based on the precise characteristics of their development in their insect hosts. Salivaria - these trypanosomes development in the anterior part of the digestive tract (e.g. the salivary glands) of their insect vector; they are said to undergo anterior station. Also, these forms typically infect new hosts as a consequence of insect biting. Stercoraria - infective forms develop in the hindgut of the insect vector; they are said to undergo posterior station. Also, these forms leave insects with the feces and infect new hosts by penetration through the skin or mucous membranes or by lesions made by vector bites.

African Trypanosomiasis

Historical review :

In 1841 G. Valentin of Berne, Switzerland saw a protozoan in the blood of a trout that moved by means of its undulating membrane; it was a trypanosome. Two years later David Gruby in Paris discovered a similar organism in frog blood and called it Trypanosoma sanguinis. Trypanosoma is derived from the Greek word 'trypano" meaning auger or screw-like and "soma" meaning body, and sanguinis comes from the Latin word for blood, "sanguis". Trypanosomes were considered mere curiosities and of no economic, medical or veterinary importance. In 1880 Griffith Evans, an English veterinarian in Punjab, India, found trypanosomes in the blood of horses, mules and camels suffering from a fatal wasting disease called surra. Inoculation of blood containing trypanosomes into healthy animals produced surra. Evans was convinced that the trypanosome (later named Trypanosoma evansi) was a parasite but he did not discover how horses, mules or camels became infected. The vector, a biting stable fly, was discovered in 1899.

Nagana

In the early 1890s the British colonial farmers of Zululand were faced with the decimation of their European breeds of cattle by a wasting disease called nagana, a word meaning in Zulu "in low or depressed spirits." Native cattle were unaffected.

Discovery of trypanosomes as cause of Nagana

In 1894, Bruce was sent to investigate Nagana in cattle. When he examined the blood of diseased cattle he described a rapidly vibrating body, lashing about among the red blood corpuscles. Bruce then went on to establish Koch's postulates for nagana: if he injected blood from cattle suffering with this disease into dogs severe wasting symptoms resulted, and abundant trypanosomes were found in the dog's blood. He wrote: "the clinical features of nagana are defined by the constant occurrence in the blood of a protozoan parasite".

Discovery of the insect vector

Tsetse Fly (*Glossina sp.*)

In 1895 Bruce discovered the vector for nagana: the blood-sucking tse-tse fly (genus: Glossina). Bruce hypothesized, and then proved, that wild game—buffalo, wildebeest, and bushbuck—were the source of infection and that transmission was the result of the bite of the tse-tse flies which infested the area in which these game animals lived.

Human Sleeping Sickness

Was observed by Arab doctors as early as 1375. In 1702 an English naval surgeon, John Atkins, described a disease ("the sleepy distemper") in Africans living along the Guinea Coast. In 1803 Thomas Winterbottom, a physician working in the colony of Sierra Leone, published an account of the "African lethargy." He recognized a telltale clinical characteristic: swelling of the cervical lymph nodes.

In May 1901 a 42-year-old Englishman who worked on the steamships plying the Gambia River came down with a fever. He was admitted to the hospital, and was treated with quinine for malaria without success. When his blood was examined no malaria parasites could be found, however, there were trypanosomes. In 1902 this trypanosome of humans was named by Joseph Everett Dutton of the Liverpool School of Tropical Medicine, T. gambiense.

Gambian Sleeping Sickness Epidemic

In 1901 a severe epidemic of sleeping sickness broke out in Uganda and so the Royal Society of London sponsored a commission headed by Bruce to investigate its cause. By 1902 the commission had discovered that the distribution of persons with Winterbottom's Sign corresponded with the distribution of sleeping sickness. Further, examination of the cerebrospinal fluid from a case with sleeping sickness by one of the members of the commission, Dr. Aldo Castellani, showed trypanosomes. He found that in the cerebrospinal fluid from 34 cases, 20 had trypanosomes, whereas in 12 control cases none were found.

Bruce suspected that the tse-tse fly was involved in the transmission of Gambian sleeping sickness. Later, when it was found that monkeys were susceptible to the disease, and a tse-tse fly fed on a human case could transmit the disease to monkeys, it was concluded: sleeping sickness is, in short, a human tse-tse fly disease.

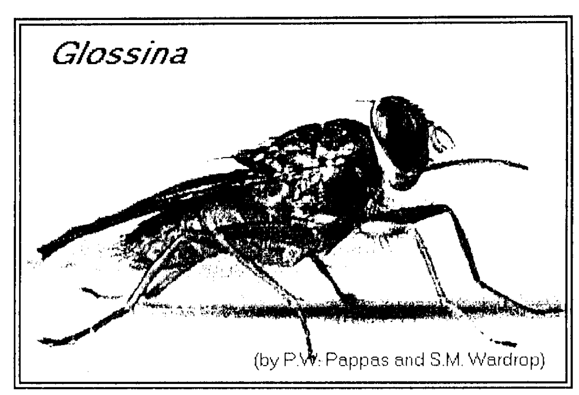
Rhodesiene Sleeping Sickness

In 1910 J. W. Stephens of the Liverpool School of Tropical Medicine discovered a new species of trypanosomes: it was from a patient with sleeping sickness who had acquired the disease in 1909 in Rhodesia, an area where T. gambiense and its vector (Glossina palpalis) did not occur. The disease was more acute and the parasites had a different morphology. Called them T. rhodesiense.

In 1912 Bruce headed another sleeping sickness commission in an area near Lake Nyssa. He found T. rhodesiense in the blood of 1/3 of the 180 game animals examined. Bruce compared the morphology of T. rhodesiense with T. brucei from cases of nagana and found them to be identical. He concluded: "T. rhodesiense is neither more nor less than T. brucei, and that the human trypanosome disease in Nyasaland is nagana". The vector was suspected to be the most abundant tse-tse, G. morsitans.

The range of African trypanosomiasis is determined by the range of the vector. Interestingly, only newly hatched tse-tse flies are competent to transmit the disease. Glossina is in fact a poor vector in nature since less than 1% of the flies are infected.

The TseTse



(by P.W. Pappas and S.M. Wardrop)

The name tsetse is derived from the noise these flies make when flying. The name itself means "fly" in Tswana. Tsetse range in size from 6-14mm. There are 31 members in the genus Glossina, 23 species and 8 sub-species. The 31 members of the Glossinidae are divided further into three sub-genera, the Glossina, Nemorhina and Austenina. The sub-genera are also called morsitans, palpalis, and fusca respectively and refer to the commonest species in each of the sub-genera. The species of the Glossina sub-genus are called the savannah flies due to their preference for this environment. It is this fact that makes them the most important vectors as the flies come into contact with both man, his animals and wild game animals.

Control of Tsetse Flies

Tse-tse fly populations may be reduced by:

1. Destruction of habitats. This may be effective by clearing vegetation near streams with riverine species (G. palpalis) for T. gambiense, but it is not possible to clear the savannah for T. rhodesiense vectors such as G. morsitans
2. Insecticides or traps with an attractant bait plus insecticide.
3. Removal of reservoirs
4. Relocation of humans and animals outside the "fly belt".

TRYPANOSOMOSIS

1-Tsetse-transmitted

Tsetse-transmitted trypanosomosis is a disease complex caused by several species of protozoan parasites of the genus Trypanosoma, transmitted cyclically by the genus Glossina (tsetse flies). Tsetse infest 10 million square kilometres and affect 37 mostly African countries. The disease infects various species of mammals but, from an economic point of view, tsetse-transmitted trypanosomosis is particularly important in cattle (in southern Africa referred to as nagana or tsetse-fly disease). It is mainly caused by Trypanosoma

congolense, T. vivax and, to a lesser extent, T. brucei brucei. Trypanosoma uniforme, T. simiae and T. suis are other, less common tsetse-transmitted species. Trypanosoma vivax is also transmitted mechanically by biting flies, as exemplified by its presence in South and Central America. Tsetse-transmitted trypanosomosis also affects humans, causing sleeping sickness, through infection with either T. brucei gambiense or T. brucei rhodesiense. Clinical signs of tsetse-transmitted trypanosomosis may include intermittent fever, oedema, and emaciation. Anaemia usually develops in affected animals and this is followed by loss of body condition, reduced productivity and often mortality. Post-mortem signs may include emaciation, enlarged lymph nodes, enlarged liver, excessive fluid in the body cavities, and petechial haemorrhages. Neither clinical nor post-mortem signs of tsetse-transmitted trypanosomosis are pathognomonic. Therefore, diagnosis must rely on direct techniques that confirm the presence of trypanosomes either by microscopic visualisation or by indirect serological techniques.

A variety of diagnostic tests are available and researchers are still trying to improve existing tests and to develop new ones. Current diagnostic tests vary in their sensitivity and specificity, the ease with which they can be applied and their cost .The choice of a particular test will be guided by economic principles and the availability of expertise, but especially by the diagnostic requirement. For example, different degrees of sensitivity and specificity are applicable to individual animals compared with herds. Similarly, the diagnostic test(s) to establish the parasitological prevalence of trypanosomosis are different from those required to establish the presence or absence of the disease in an area. Reliable diagnosis may be achieved by combining appropriate diagnostic tests. Reliable interpretation of results from diagnostic tests will depend, to a large extent, on proper sample selection/collection, the sample size, and the way the diagnostic tests are conducted.

1-Identification of the agent

Parasite detection techniques are highly specific, but their sensitivity is relatively low (i.e. the proportion of false-negative results recorded is high). Sensitivity is especially low when results are considered at the individual animal level rather than the herd level. Due to this low sensitivity, the apparent parasitological prevalence of trypanosomosis is generally lower than the true parasitological prevalence. The low diagnostic sensitivity also makes it difficult to detect trypanosomosis when present at low parasitological prevalence and it is impossible to establish the absence of the disease with a high degree of confidence. Moreover, in areas where trypanocidal drugs are used extensively, parasites may not be detected.

Several parasite detection techniques are available, each with varying sensitivity. The choice will depend on the laboratory facilities available and the aim of the diagnosis.

Direct examination techniques

The simplest techniques are examination of wet, thick or thin films of fresh blood, usually obtained from the ear vein, jugular vein or the tail.

a) Wet blood films

These are made by placing a drop of blood (about 2 µl) on a clean microscope slide and covering with a cover-slip (22 x 22 mm). The blood is examined microscopically using a x40 objective lens with reduced condenser aperture or by phase-contrast. Approximately 50-100 fields are examined. Trypanosomes can be recognised by their movement among the red blood cells (RBCs).

The method is simple, inexpensive and gives immediate results. Depending on the trypanosome size and movements a presumptive diagnosis can be made of the trypanosome species. Final confirmation of the species is made by the examination of the stained preparation.
The diagnostic sensitivity of the method is generally low but depends on the examiner's experience and the level of parasitaemia. Sensitivity can be improved significantly by lysing the RBCs before examination using a haemolytic agent such as sodium dodecyl sulfate (SDS).

b) Thick blood films

These are made by placing a drop of blood (5-10 µl) on a clean microscope slide and spreading it over an area of approximately 2 cm in diameter, using the corner of another slide. The thickness of the resultant film should be such that, when dry, the figures on a wristwatch dial can just be read through it. The film is dried thoroughly by rapidly waving in the air and, without fixation, is dehaemoglobinised by immersion in distilled water for a few seconds and dried before staining. A dry smear should be kept dry and protected from dust, heat, flies and other insects. It is stained for 30 minutes with 4% diluted Giemsa stain in phosphate buffered water, pH 7.2. Staining time and stain dilution may vary with stain and individual technique. Therefore, it is important to start with the manufacturer's directions and to vary staining time and stain concentration to obtain the optimal result. The stained smear is then washed with buffered water and examined with a x50 or x100 oil-immersion-objective-lens.
The method is simple and relatively inexpensive, but results are delayed because of the staining process. Trypanosomes are easily recognised by their general morphology, but may be damaged during the staining process. This may make it difficult to identify the species.

c) Thin blood smear films

Thin blood smears are made by placing a small drop of blood (about 5 µl), for example from a microhaematocrit capillary tube, on a clean microscope slide approximately 20 mm from one end (allowing for space to apply the thick smear) and spreading with the edge of another slide. This slide is placed at an angle of approximately 30° to the first slide and drawn back to make contact with the blood droplet. The blood is allowed to run along the edge of the spreader, which is then pushed to the other end of the slide in a fairly rapid but smooth motion. If the correct amount of blood is used, the slide should be covered with a film of blood with no surplus before the end of the slide is reached. Ideally, thin films should be prepared so that the RBCs are fairly close to each other but not overlapping. The slide is dried quickly by waving in the air and protected from dust, flies and other insects. The slide is fixed for 3 minutes in methanol, and stained as for thick blood smears. After staining, the slide is washed gently under tap water and allowed to dry. A variation of this method is to fix in methanol for 2 minutes, apply May/Grünwald stain for 2 minutes, then add an equal volume of buffered water, pH 7.2, leave for a further 8 minutes and drain off. Approximately 50-100 fields of the stained thin smear are examined, with a x50 or x100 oil-immersion objective lens, before the specimen is considered to be negative. Even after a trypanosome has been detected, approximately 20 extra fields are investigated to determine if more than one species is present.
The technique described above can also be used for biopsy samples of lymph obtained from punctured lymph nodes.
Usually, both a thin and thick smear is made from the same sample. Thick smears contain more blood than thin smears and, hence, have a higher

diagnostic sensitivity. Thin smears on the other hand allow Trypanosoma species identification. Trypanosome species can be identified by the following morphological characteristics.

Trypanosoma vivax: 20-27 µm long, undulating membrane is not obvious, free flagellum present at the anterior end, posterior end rounded, kinetoplast large and terminal.

Trypanosoma brucei is a polymorphic trypanosome species. Two distinctly different forms can be distinguished, i.e. a long slender form and a short stumpy form. Often, intermediate forms, possessing characteristics of both the slender and stumpy forms, are observed. The cytoplasm often contains basophilic granules in stained specimens.

Trypanosoma brucei (long slender form): 17-30 µm long and about 2.8 µm wide, undulating membrane is conspicuous, free flagellum present at the anterior end, posterior end pointed, kinetoplast small and subterminal. Trypanosoma brucei (short stumpy form): 17-22 µm long and about 3.5 µm wide, undulating membrane is conspicuous, free flagellum absent, posterior end pointed, kinetoplast small and subterminal.

Trypanosoma congolense: 8-25 µm (small species), undulating membrane not obvious, free flagellum absent, posterior end rounded, kinetoplast is medium sized and terminal, often laterally positioned. Although T. congolense is considered to be monomorphus, a degree of morphological variation is sometimes observed. Trypanosoma theileri: 60-70 µm (large species), undulating membrane is conspicuous, long free flagellum present, posterior end pointed, kinetoplast is large and positioned near the nucleus. Trypanosoma theileri is normally nonpathogenic, but its presence can confuse the parasitological diagnosis.

- Parasite concentration techniques

The probability of detecting trypanosomes in a sample from an infected animal depends largely on the amount of blood examined and the level of parasitaemia. The amount of blood examined with direct examination techniques is low and parasites are often very scanty in the blood of an infected animal. Both of these factors contribute to the low sensitivity of direct examination techniques. Sensitivity can be improved by increasing the volume of blood to be examined and by concentrating the trypanosomes.

a) Microhaematocrit centrifugation technique (Woo method)

The microhaematocrit centrifugation technique, or the Woo method, is widely used for the diagnosis of animal trypanosomosis. It is based on the separation of the different components of the blood sample depending on their specific gravity. The method is as follows:

i) Fresh, usually ear vein blood (about 70 µl) is collected into heparinised capillary tubes (75 x 1.5 mm).

ii) One end of the capillary tube is sealed with cristaseal or by heating, ensuring that the column of blood is not charred by the flame.

iii) The sealed capillary tubes are placed in a microhaematocrit centrifuge with the sealed ends pointing towards the outside. To ensure good balance, the tubes are loaded symmetrically.

iv) The rotary cover is screwed on and the centrifuge lid is closed.

v) The capillary tubes are centrifuged at 9000 g for 5 minutes.

vi) A tube carrier is made from a slide on which two pieces of glass 25 x 10 x 1.2 mm have been fixed, 1.5 mm apart, to form a groove.

vii) The tube is placed in the groove, a cover-slip is placed on top and the interface is flooded with water.

viii) The plasma/white blood cell interface (buffy coat) is examined by slowly rotating the tube. Trypanosome movement can first be detected using the x10 objective lens with reduced condenser aperture; the trypanosomes can be seen more clearly using the x40 objective lens preferably with a long working distance to allow adequate depth of focus through the capillary tube.

The microhaematocrit centrifugation technique is more sensitive than the direct examination techniques. Identification of trypanosome species is difficult. As the specific gravity of T. congolense is similar to that of RBCs, parasites are often found below the buffy coat in the RBC layer. To improve the separation of RBCs and parasites, and increase the sensitivity for T. congolense, the specific gravity of RBCs can be increased by the addition of glycerol.

A modification of the Woo method is the quantitative buffy coat method (QBC). The method has been used for the diagnosis of T. b. gambiense infections. The method is probably too expensive for the routine large-scale use in animal trypanosomosis surveys.

b)Dark-ground/phase-contrast buffy coat technique

The buffy coat technique or Murray method represents an improved technique for the detection of trypanosomes and is widely used. It is carried out following steps i to v above, after which the capillary tube is cut, with a diamond tipped pencil, 1 mm below the buffy coat, to include the top layer of RBCs. The buffy coat and the uppermost layer RBCs are extruded on to a clean microscope slide and covered with a cover-slip (22 x 22 mm). Approximately 200 fields of the preparation are examined for the presence of motile trypanosomes with a dark-ground or a phase-contrast microscope with a x40 objective lens. Trypanosome species can be identified by reference to the following criteria:

Trypanosoma vivax: Large, extremely active, traverses the whole field very quickly, pausing occasionally.

Trypanosoma brucei: Various sizes, rapid movement in confined areas.

Trypanosoma congolense: Small, sluggish, adheres to RBCs by anterior end.

Trypanosoma theileri: More than twice the size of pathogenic trypanosomes, tends to rotate

As with the microhaematocrit centrifugation technique, the buffy coat technique is more sensitive than direct examination techniques. The sensitivity of the buffy coat method can be improved by using the buffy coat double centrifugation technique). A total amount of 1500-2000 µl of blood is centrifuged, after which the buffy coat is aspired into a microhaematocrit capillary tube and centrifuged again. The buffy coat is examined. Compared with the microhaematocrit centrifugation technique, the buffy coat technique has the added advantage that preparations can be fixed and stained for more accurate identification of species and for retention as a permanent record.

Both the microhaematocrit centrifugation and buffy coat techniques give direct results and can be used for screening large numbers of animals. They require specialised equipment and an electricity supply making the test more expensive compared with the examination of the wet blood film. However, this is compensated for by increased sensitivity. Both parasite concentration techniques rely on the detection of motile, live, trypanosomes. As trypanosomes are heat-sensitive, samples collected in capillary tubes should be kept cool and the microhaematocrit centrifuge should not be allowed to overheat.

The microhaematocrit centrifugation and buffy coat techniques are particularly useful in that the packed cell volume (PCV) can be assessed at the same time.

To determine the packed cell volume after centrifugation, the microhaematocrit capillary tube (containing ear vein or jugular vein blood) is placed in a haematocrit reader. The length of the packed RBC column is expressed as a percentage of the total volume of blood. Measuring the PCV is useful for determining the degree of anaemia. Anaemia can be caused by factors other than tsetse-transmitted trypanosomosis. It remains, however, one of the most important indicators of trypanosomosis in cattle. As trypanosomosis is a herd problem, the PCV-profile of a herd is influenced by the number of trypanosome-infected animals and can be used to indicate differences in disease_challenge.

c) Anion exchange

The miniature anion-exchange chromatography technique (m-AECT) is widely used for the diagnosis of human sleeping sickness caused by T. b. gambiense. Blood is passed through a diethyl amino-ethyl (DEAE)-cellulose column equilibrated with a phosphate buffered saline (PBS) solution of an ionic strength suited to the blood of the animal species under examination. As the RBCs are more negatively charged than the trypanosomes, they are held in the column and the trypanosomes pass through with the eluate, which is collected, centrifuged to concentrate the trypanosomes and examined under the microscope.

Large volumes of blood can be examined from each animal and, therefore, the method has high sensitivity. However, the technique is cumbersome and is not suitable for the examination of a large number of animals.

d) In-vitro cultivation

A procedure for the in-vitro cultivation of T. brucei has been described, but success has been irregular over many years. Moreover, the method needs sophisticated equipment, produces results after a considerable delay and is certainly not suitable for large-scale use. A recently described kit (KIVI) for in-vitro isolation of trypanosomes has proven to be promising in isolating and amplifying T. b. gambiense in humans, domestic and game animals. The test's value in isolating T. congolense and T. vivax is still unknown. As it is based on the cultivation of procyclic forms of trypanosomes, species differentiation is not possible.

- Animal inoculation

The subinoculation of blood into rodents, usually mice or rats, is particularly useful in revealing subpatent infections. The laboratory animals are injected intraperitoneally with 0.2-5 ml (depending on the size of the rodent) of freshly collected blood. They are bled three times a week for at least 2 months. Collected blood is examined using the wet film-method.

Animal inoculation is more sensitive than direct examination of the wet blood film. Nevertheless, the method is not practical; it is expensive and diagnosis is not immediate. The method is highly sensitive in detecting T. brucei infections. However, some T. congolense strains are not easily transmitted and T. vivax rarely infects laboratory rodents.

- Tests to detect trypanosomal antigen

An antigen-detection enzyme-linked immunosorbent assay (antigen ELISA) for trypanosomosis has been described. Field evaluations of the test have given inconsistent results. Therefore, additional work is needed to discover and overcome the cause of those inconsistencies before the test can be used in the routine diagnosis of trypanosomosis.

- DNA amplification tests

A polymerase chain reaction (PCR) method has been developed as a tool for the diagnosis of infections with African trypanosomes in humans and animals, as well as tsetse flies. Specific repetitive nuclear DNA sequences can be amplified for T. vivax and each of the five T. congolense subgroups. A common primer set is available for detection of the three T. brucei subspecies. The primer sets available for different trypanosome species, subspecies and subgroups are referred to as follows: brucei subspecies: TBR1 and TBR2 congolense (Savannah subgroup); TCN1 and TCN2 congolense (Forest subgroup); TCF1 and TCF2 congolense (Kenya Coast subgroup); TCK1 and TCK2 vivax: TVW1 and TVW2.

Standard PCR amplifications are carried out in a reaction mixture containing Tris-HCl, MgCl2, KCl, each of the four deoxyribonucleotide triphosphates, primers, DNA template and Taq DNA polymerase. Samples are incubated during several cycles at varying temperatures. The PCR products are electrophoresed through agarose. Gels are stained with ethidium bromide.

The procedure is extremely sensitive, but false-positive results may occur as a result of contamination of samples with other DNA. The test requires specialised equipment and highly trained personnel, so it is not suitable for use in many laboratories. False-negative results may occur when the specificity of the primers is too high so that not all types of a particular trypanosome species are recognised. Sample collection has been simplified by adapting the test using blood spotted on to filter paper. A large number of samples can be processed at one time, making it potentially suitable for large-scale surveys. However, at the moment, the cost of PCR analyses are prohibitive for the routine use of the test.

2. Serological tests

Several antibody detection techniques have been developed to detect trypanosomal antibodies for the diagnosis of animal trypanosomosis, with variable sensitivity and specificity. The methods of choice are the indirect fluorescent antibody test (IFAT) and the trypanosomal antibody-detection ELISA. The development and testing of specific recombinant antigens could, in the near future, allow the detection of species-specific antibodies.

a) Indirect fluorescent antibody test

The original method for this test has been replaced by a new technique for the preparation of trypanosomal antigens,which involves fixation of live trypanosomes using a mixture of 80% cold acetone and 0.25% formalin in normal saline.

Test procedure

i) Prepare thin smears from heavily parasitaemic blood or from a trypanosome suspension. Air-dry and fix in acetone for 5 minutes.

ii) Mark circles of 5 mm diameter on glass slides using nail varnish.

iii)Using a pipette, place a test serum, diluted 1/40, in each circle, ensuring that the area in each circle is completely covered.

iv) Incubate the antigen/test serum preparation at 37°C for 30 minutes in a humid chamber.

v) Wash the preparation three times in PBS for 5 minutes each time at 4°C, with gentle agitation. Air-dry the slides.

vi) Apply conjugate: rabbit or goat anti-bovine IgG (for tests on bovine sera) conjugated to fluorescein isothiocyanate.

vii) Incubate and wash as above. Rinse in distilled water. Air-dry the slides.

viii) Mount the slides in PBS or buffered glycerol and examine for fluorescence.

b) Antibody-detection enzyme-linked immunosorbent assay

The original antibody ELISA has recently been further developed for use in large-scale surveys of bovine trypanosomosis.

Test procedure

•Minor modifications are made to the original procedure)

i) Dilute antigen in 50 mM carbonate/bicarbonate buffer, pH 9.6, and coat microtitre plates overnight at 4°C.

ii) Wash microtitre plates four times in 2 mM PBS, pH 7.4, containing 0.05% Tween 20.

iii)Dilute sera at 1/400 in PBS, 10 mM, pH 7.4, containing 0.04% Tween 20. Punch 6 mm disks from dried bloodspots on filter paper and elute in 2.0 ml PBS for 2.5 or 5 hours at room temperature or overnight at 4°C.

iv) Incubate diluted sera or bloodspots with anti-bovine IgG peroxidase conjugate in microtitre plate wells at 37°C with orbital shaking for 45 and 30 minutes, respectively.

v) Wash microtitre plates five times in 2 mM PBS, pH 7.4, containing 0.05% Tween 20.

vi)Incubate for 10 minutes in tetramethylbenzidine at 37°C with orbital shaking followed by an H2SO4 quench.

vii)Read absorbance at 450 nm.

The standard antigen for trypanosomosis antibody tests is derived from bloodstream-form trypanosomes. Antigens are prepared as a soluble fraction of trypanosomes purified by DEAE anion-exchange chromatography of parasites from whole blood of infected rats, with lysis using seven freeze/thaw cycles and centrifugation at 10,000 g for 30 minutes. Antigens obtained from in-vitro propagated procyclic trypanosome forms can also be used. Both the IFAT and antibody-detection ELISA have been adapted for the analysis of blood samples collected on filter paper. Blood contained in one heparinised microhaematocrit centrifuge capillary tube is extruded on to a filter paper. Samples are air-dried out of direct sunlight and placed in a plastic bag with self-indicating silica gel desiccant. The bag is sealed and should be kept as cool as possible until specimens are refrigerated or frozen.

Each ELISA-microplate is run with strong positive, weak positive and negative reference sera, which are required to comply with pre-set values for quality assurance. The absorbance of each ELISA-sample tested is expressed as a percentage (percentage positivity, PP) of the strong positive reference standard. Results are, therefore, quantifiable. The cut-off value is determined using known positive and negative field samples.

Both antibody-detection tests have high sensitivity and specificity. Their species specificity is generally low. They detect immune responses to current and past infections and can, therefore, only provide a presumptive diagnosis of active infection.

Immunodiagnosis needs expensive, sophisticated equipment and expertise, which is not always available. It has to be performed in specialised laboratories and there is a substantial delay between the actual sampling and the availability of the results. Nevertheless, the antibody ELISA lends itself to a high degree of automation and standardisation. Sample collection and storage is made easy through the use of filter papers. All of these factors make the antibody ELISA a very useful test for large-scale surveys to determine the distribution of tsetse transmitted trypanosomosis.

c) Card agglutination test

This test was originally developed for the diagnosis of human sleeping sickness (T. b. gambiense) and has been further adapted for the detection of antibodies against T. evansi.

2 -Non tsetse-transmitted trypanosomoses

There are strains of two African trypanosome species that have adapted to mechanical transmission by biting flies in various animal species, or to life in tissue fluids and venereal transmission in horses and donkeys(T.evansi and T.equiperdium).

Trypanosoma evansi

T. evansi has almost certainly arisen from T. brucei brucei by adaptation to mechanical transmission by biting flies, and it remains very closely related to T. brucei. It has lost the capability of being cyclically transmitted by tsetse flies and in the process has become almost monomorphic, the great majority of the parasites being indistinguishable from the long slender forms of T. brucei. It has spread far outside the tsetse regions of sub-Saharan Africa and is at present known to occur throughout the Sahel region of Africa, in North Africa, most Near and Far East Asian countries (including southern Siberia, China and Indonesia) and many countries in Latin America, from Argentina in the south to at least Venezuela and Colombia in the north. The situation in central America is not well defined, but in the past the parasite has been reported from at least one country, Panama.

T. evansi is the cause of the most important parasitic disease of camels, and is also highly pathogenic for horses (and mules and donkeys) and for dogs. It is also of considerable economic importance in Asian (domestic) buffalo, and to a less extent in some countries in cattle as well. Many other host species, wild as well as domestic, have been found naturally infected. The most common name for this disease is surra, of Indian origin, now generally accepted, but in Africa and Latin American countries there are various other names .Apart from the name surra, which originates from India, the disease is known in many Arabic-speaking parts of Africa as el debab, and many other local names exist, e.g. gufar in the Sudan. It probably occurs wherever camels are reared.

Surra in camels is usually a chronic wasting disease.Striking clinical symptoms develop a month or so after infection, with acute bouts of fever, associated with dullness, lack of appetite and lachrymation, coinciding with peaks of high parasitaemia. Gradually the animal loses condition, the hump shrinks and progressive weakness becomes noticeable. Oedemas may occur. Pregnant animals often abort. The disease is usually fatal, sometimes rather quickly, a few weeks or more often a few months after the onset of the disease; the evolution is much more often chronic, and may last as long as two or three years. In chronic cases it is usually very difficult to find parasites in the blood by direct means.

The most important mechanical vectors of surra in camels are probably tabanids; hippoboscids (Hippobosca camelind) are also suspected, but so far there is no formal proof of their vector role.

A) Surra

Murrina, Mal de Caderas, Derrengadera

Surra is a protozoal disease that can affect most mammals but is generally more severe in horses. In endemic areas, the economic cost of this disease can be considerable; in Africa, Asia, and South America, surra causes the death of thousands of animals each year.

Etiology

Surra is caused by infection with the protozoal parasite Trypanosoma evansi.

Species affected

T. evansi is pathogenic in most domesticated animals and some wild animals. Horses, mules, donkeys, camels, llamas, deer, cattle, buffalo, cats, and dogs are commonly affected. Asymptomatic, mild, or chronic disease has been seen in sheep, goats, elephants, and pigs. Outbreaks have also been reported in captive tigers and jaguars in India.

The main host species varies with the geographic region: camels are most often affected in the Middle East and Africa, horses in South America, and horses, mules, buffalo, and deer in China. In Southeast Asia, surra is seen mainly in horses, cattle, and buffalo. Capybara are reservoir hosts and vampire bats are both reservoir hosts and vectors in South and Central America.

Geographic distribution

Surra is endemic in China, the Indian subcontinent, Southeast Asia, northern Africa, the Middle East, South America, the Philippines, Bulgaria, parts of the former U.S.S.R., and parts of Indonesia.

Transmission

T. evansi is transmitted mechanically by biting flies in the genera Tabanus, Lyperosia, Stomoxys, and Atylotus. Species of Tabanus appear to be the most significant vectors. There is no intermediate host. Vampire bats can spread infections in South and Central America. Carnivores may become infected after feeding on infected meat.

Transmission in milk and during coitus has also been documented.

Incubation period In the Equidae, the incubation period varies from 5 to 60 days.

Clinical signs

Surra may be a subacute, acute, or chronic disease. In horses, donkeys, and mules, typical clinical signs include an intermittent fever, lethargy, weakness, weight loss, petechiae on the mucus membranes, and extravasation of blood at the mucocutaneous junctions of the nostrils, eyelids, and anus. Urticaria, jaundice, or anemia may be apparent, and there may be edema in the legs, abdomen, and brisket. Exudation, alopecia, necrosis, or ulceration may be seen at the coronary bands and the lymph nodes may be enlarged. In South America, horses with Mal de Caderas have anemia, emaciation, gradually progressive paresis of the hindquarters, and sometimes urticaria. In the Equidae, surra is often fatal within two weeks to four months.

In cattle and buffalo, surra is typically chronic, but may also be mild or asymptomatic. In chronic infections, the clinical signs may include an intermittent fever, anemia, emaciation, edema of the brisket, and paresis in the

hind legs. Abortions may be seen in buffalo. Some animals die during the first six months, but most recover and become carriers.

Infections in cats and dogs are usually acute and fatal. Common symptoms in dogs include an intermittent fever and edema of the head, legs, and abdominal wall. Dogs may also have nervous signs that resemble rabies. In deer, surra is usually chronic and is characterized by edema, anemia, emaciation, and nervous signs. Camels experience symptoms similar to those seen in horses, but chronic infections with wasting and anemia are more common. Infections are usually asymptomatic or mild in pigs and chronic in goats.

Post mortem lesions

The post-mortem lesions may include emaciation of the carcass, anemia, and petechiae on some internal organs. Hydrothorax and ascites are sometimes seen. The spleen and lymph nodes may be enlarged.

Morbidity and Mortality

The severity of disease can vary with the strain of trypanosome and with host factors, including stress, concurrent infections, and general health. Outbreaks of surra may be associated with the movement of infected animals into disease-free areas or susceptible animals into endemic areas. In some outbreaks, morbidity up to 50–70% and comparable mortality can be seen. In China, the average mortality rate in horses is 41%, and the average mortality rate in cattle and buffalo 28%. In general, mortality is high in horses, dogs, and cats and lower in

cattle, buffalo, and other species. In endemic areas, surra can be treated with anti-protozoal drugs.

Diagnosis

Clinical

Typical symptoms of surra include fever, anemia, weight loss, edema, and enlargement of the lymph nodes and spleen.

Differential diagnosis

In horses, the differential diagnosis includes African horse sickness, equine viral arteritis, equine infectious anemia, and chronic parasitism. In dogs, rabies must also be considered.

Laboratory tests

Surra can be diagnosed by finding T. evansi in the blood, lymph nodes, skin exudates, liver, lungs, or kidney. For microscopic examination, thin films and smears are stained with Giemsa or another Romanowsky-type stain, and thick films by Field's method. The parasites are often difficult to find by direct examination; detection may be improved by the hematocrit tube centrifugation technique or mini anion-exchange chromatography. Morphologically, T. evansi cannot be distinguished from T. equiperdum or some forms of T. brucei. T. evansi antigens can be detected with a latex agglutination test or enzyme-linked immunosorbent assays (ELISAs). A polymerase chain reaction (PCR) test has also been published and a reverse indirect hemagglutination

test is being tested in China. Serology can be valuable, but may not distinguish between current and past infections. Published serological

techniques include ELISAs, fluorescent antibody tests, and a modified card–agglutination test. Animal inoculation studies in rats or mice are occasionally used; they are very sensitive but time–consuming.

Samples to collect

Before collecting or sending any samples from animals with a suspected foreign animal disease, the proper authorities should be contacted. Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease. To detect trypanosomes, several thick and thin blood films should be made during the febrile phase and air–dried. . Thick and thin slides may be also made from needle biopsies of the prescapular or precrural lymph nodes, and smears from any skin exudates. Post–mortem, impression smears should be collected from the lungs, liver, and kidney. In live animals, repeated sampling may be necessary to detect the organism. Approximately 10 ml blood should also be collected into heparin or EDTA, with antibiotics added. Another 25 ml of blood should be taken for serology. These samples should be transported cold, with wet ice or gel packs.

Quarantine and Disinfection

Trypanosomes cannot survive for long outside the host, and T. evansi disappears quickly from the carcass after death. Controlling arthropod vectors and preventing their access to host species is important in preventing new infections. Flies are most infective during the first few

minutes after feeding on an infected host; after 8 hours, they no longer transmit the parasites. The movement of potentially infected animals must also be restricted.

Public health

There is no evidence that T. evansi is a hazard to human health.

B)DOURINE

(el Dourin, Mal de Coit,Covering Disease)

Definition

Dourine is a chronic trypanosomal disease of Equidae. The disease is transmitted almost exclusively by coitus and is characterized by edematous lesions of the genitalia, nervous system involvement, and progressive emaciation.

Etiology

Dourine is caused by Trypanosoma equiperdum ,a protozoan parasite related morphologically and serologically to T. brucei, T. rhodesiense, and T. gambiense (of the subgenus Trypanozoon of the Salivarian section of organisms of the pathogenic genus Trypanosoma). Different strains of the parasite vary in pathogenicity.

Host Range

Dourine is typically a disease of horses and donkeys. Positive CF tests have been obtained from zebras, although it has not been shown that zebras can be infected with T. equiperdum or transmit the disease. The organism has been adapted to a variety of laboratory animals.

Improved breeds of horses seem to be more susceptible to the disease. The disease in these animals often progresses rapidly and involves the nervous system. In contrast, native ponies and donkeys often exhibit only mild signs of the disease. Infected male donkeys, which may be asymptomatic, are particularly dangerous in the epidemiology of the disease, for they may escape detection as carriers.

Geographic Distribution

Once widespread, this disease has been eradicated from many countries. It is currently present in most of Asia, southeastern Europe, South America, and in northern and southern Africa.

Transmission

This venereal disease is spread almost exclusively by coitus. Organisms are present in the urethra of infected stallions and in vaginal discharges of infected mares. The organism may pass through intact mucous membranes to infect the new host. Infected animals do not transmit the infection with every sexual encounter, however. As the disease progresses, trypanosomes periodically disappear from the urethra or vagina; during these periods, the animals are noninfective. Noninfective periods may last for weeks or months and are more likely to occur in the later stages of the disease. Thus, transmission is most likely early in the disease process.

It is possible for mares to become infected and pregnant after mating with an infected stallion. Foals born to infected mares may be infected. It is unclear if this occurs in utero or during birth. Because trypanosomes may occur in the milk of infected mares, these foals may be infected per os during birth or by ingestion of infected milk. Foals infected in this way may transmit the disease when mature and develop a lifelong positive CF titer. This method of disease transmission is rare, however. Some foals may acquire passive immunity from colostrum of infected mares without becoming actively infected; in such foals, the CF titer declines, and the animal becomes seronegative by 4 to 7 months of age. Although the possibility of noncoital transmission remains uncertain, it is supported by sporadic infections in sexually immature equids.

Incubation Period

The incubation period is highly variable. Clinical signs usually appear within a few weeks of infection but may not be evident until after several years.

Clinical Signs

Clinical signs vary considerably, depending on the virulence of the infecting strain, the nutritional status of the infected animal, and the presence of other stress factors. The strain prevalent in southern Africa (and formerly in the Americas) is apparently less virulent than the European, Asian, or north African strains and produces an insidious, chronic disease. In some animals, clinical signs may not be apparent for up to several years (so-called latent infection). Clinical signs may be precipitated by stress in these animals. In mares, the first sign of infection is usually a small amount of vaginal discharge, which may remain on the tail and hindquarters. Swelling and edema of the vulva develop later and extend along the perineum to the

udder and ventral abdomen. There may be vulvitis and vaginitis with polyuria and other signs of discomfort such as an elevated tail. Abortion is not a feature of infection with mild strains, but significant abortion losses may accompany infection with a more virulent strain.

In stallions, the initial signs are variable edema of the prepuce and glans penis, spreading to the scrotum and perineum and to the ventral abdomen and thorax. Paraphimosis may be observed. The swelling may resolve and reappear periodically. Vesicles or ulcers on the genitalia may heal and leave permanent white scars (leukodermic patches). Transient cutaneous plaques are a feature of the disease in some locations and strains but not others. When they occur, they are pathognomonic. Conjunctivitis and keratitis are often observed in outbreaks of dourine and may be the first signs noted in some infected herds.

Nervous disorders may be seen soon after the genital edema or may follow by weeks or months. Initially these signs consist of restlessness and the tendency to shift weight from one leg to another followed by progressive weakness and incoordination and ultimately by paralysis and recumbency. Anemia and emaciation sometimes accompany development of clinical signs even though the appetite remains unaffected.

Dourine is characterized by stages of exacerbation, tolerance, or relapse that may vary in duration and occur several times before death or recovery. The course of the disease may last several years after infection with a mild strain. Experimentally, horses have survived for up to 10 years after infection. The course is apparently more acute in the European and Asian forms of the disease in which the mortality rate is higher.

Gross Lesions

Anemia and cachexia are consistent findings in animals that have succumbed to dourine. Edema of the genitalia and ventral abdomen become indurated later in the course of the disease. Chronic lymphadenitis of most lymph nodes may be evident. Perineural connective tissue becomes infiltrated with edematous fluid in animals with nervous signs, and a serous infiltrate may surround the spinal cord, especially in the lumbar or sacral regions.

Mortality

Although the course of the disease may be long, it is usually fatal. Uncomplicated dourine does not appear to be fatal unless the nervous system is involved. The progressive debilitation associated with the neurological manifestation of the disease predisposes infected animals to a variety of other conditions. Because of the long survival time in some experimental cases, reports of recovery from dourine should be regarded with skepticism.

Diagnosis

Field Diagnosis

Diagnosis on physical signs is unreliable because many animals develop no sign. When signs are present, however, they are suggestive of a diagnosis of dourine. If "silver dollar plaques" occur, they are pathognomonic for dourine.

Specimens for the Laboratory

Detection of trypanosomes is highly variable and is not a reliable means for diagnosis of dourine. The following specimens should be submitted: serum, whole blood in EDTA, and blood smears.

Laboratory Diagnosis

A reliable complement-fixation test (CFT) has been the basis for the successful eradication of dourine from many parts of the world. The antigen used in the CFT is group-specific, leading to cross-reactions with sera of horses infected with T. brucei, T. rhodesiense, or T. gambiense. The test is therefore most useful in areas where these parasites do not occur. Indirect fluorescent antibody, card agglutination, and enzyme-linked immunosorbent assay test (ELISA) have also been developed for dourine but have not replaced the CFT.

Differential Diagnosis

The perineal and ventral abdominal edema characteristic of dourine may also be seen in horses with anthrax. These signs may also resemble infection with equine infectious anemia or equine viral arteritis. Coital exanthema and purulent endometritis (as occurs in contagious equine metritis) should also be considered.

Treatment

Although there are reports of successful treatment with trypanocidal drugs (e.g., suramin at 10 mg/kg IV, quinapyramine dimethylsulfate at 3-5 mg/kg SC), treatment is more successful when the disease is caused by the more virulent (European) strains of the parasite. In general, treatment is not recommended for fear of continued dissemination of the disease by treated animals (1,6). Treatment may result in inapparent disease carriers and is not recommended in a dourine-free territory.

Vaccination

Immunity to trypanosomiasis is complicated. T. equiperdum has the ability periodically to replace major surface glycoprotein antigens, which is a strategy supporting chronic infections. No method of immunization against dourine exists at present.

Control and Eradication

The most successful prevention and eradication programs have focused on serologic identification of infected animals. Infected animals should be



humanely destroyed or castrated to prevent further transmission of the disease. Some geldings may still show service behavior and constitute a risk. All equids in an area where dourine is found should be quarantined and breeding should be stopped for 1 to 2 months while testing continues.

Sanitation and disinfection are ineffective means of controlling the spread of dourine because the disease is normally spread by coitus.

Public Health

Humans are not susceptible to infection with T. equiperdum.

Treatment of Animal Trypanosomosis

Drug	Other Names	Animal	Trypanosoma	Main Action
Diminazene aceturate	Berenil, babesin (as the delactate salt), ganaseg	Cattle	vivax, congolense, brucei	Curative (with the possible exception of brucei)
		Dogs	evansi, congolense, brucei	
Homidium bromide	Ethidium bromide	Cattle	vivax, congolense, brucei	Curative
		Equids	vivax	
Homidium chloride	Babidium chloride, ethidium chloride, novidium chloride		As for the bromide salt	
Isometamidium	Samorin M & B 4180	Cattle	vivax, congolense	Curative and prophylactic
Prothidium		Cattle	vivax, congolense	Curative and prophylactic
Quinapyramine sulfate	Antrycide sulfate	Cattle	vivax, congolense, brucei, evansi	Curative
		Horses	brucei, evansi, equiperdum	
		Camels	evansi	
		Pigs	simiae	
		Dogs	congolense brucei	
Quinapyramine	Antrycide prosalt	Cattle, pigs	vivax, congolense, simiae	Prophylactic
Suramin	Moranyl, naganol	Horses	brucei, evansi, equinum	Curative
	Antrypol	Camels	evansi	
	Bayer 205, naphuride, germanin	Dog	brucei, evansi	

شركة ترى إم التجارية

استيراد تصدير –توكيلات تجارية

وكلاء شركة بترا المصرية الاردنية (بترا)

١ - أديمول ← أ د ٣ هـ عالى التركيز ٤٠/٢٠/١٠٠

٢ - سولافيت ك ٣ ← ك ٣ سائل ١٠%

٣ - سيلفرول هـ١٠% + سيلنيوم

٤ - بروموسول ← احماض أمينيه +فيتامينات

٥- أ د ٣ هـ + جـ

٦- أسكوربين فيتامين ج٢٥% سائل

٧- فيتامين د ٣

٨ - فيروسيللين

٩- ساين اب ← مدر بول ومنشط كبد سائل

١٠- الفاكيور ← مضاد فطر وسموم سائل

٩عمارات بنك الاسكان –كفرالشيخ امام فندق الرياضين
تليفاكس ٢٢٠٦٨٥ /٠٤٧